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(57) Abstract

By this invention, compositions and methods of use related to β–ketoacyl–ACP synthase of special interest are synthases obtainable from *Cuphea* species. Amino acid and nucleic acid for synthase protein factors are provided, as well as methods to utilize such sequences in constructs for production of genetically engineered plants having altered fatty acid compositions. Of particular interest is the expression of synthase protein factors in conjunction with expression of plant medium–chain acyl–ACP thioesterases for production of increased levels and/or modified ratios of medium–chain fatty acids in oils of transgenic plant seeds.

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PLANT FATTY ACID SYNTHASES AND USE IN IMPROVED METHODS FOR PRODUCTION OF MEDIUM-CHAIN FATTY ACIDS

INTRODUCTION

Field of Invention

The present invention is directed to genes encoding plant fatty acid synthase enzymes relevant to fatty acid synthesis in plants, and to methods of using such genes in combination with genes encoding plant medium-chain preferring thioesterase proteins. Such uses provide a method to increase the levels of medium-chain fatty acids that may be produced in seed oils of transgenic plants.

Background

Higher plants synthesize fatty acids via a common metabolic pathway. In developing seeds, where fatty acids attached to triglycerides are stored as a source of energy for further germination, the fatty acid synthesis pathway is located in the plastids. The first step is the formation of acetyl-ACP (acyl carrier protein) from acetyl-CoA and ACP catalyzed by a short chain preferring condensing enzyme, ß-ketoacyl-ACP synthase (KAS) III. Elongation of acetyl-ACP to 16- and 18- carbon fatty acids involves the cyclical action of the following sequence of reactions: condensation with a two-carbon unit from malonyl-ACP to form a longer ß-ketoacyl-ACP (ß-ketoacyl-ACP synthase), reduction of the

keto-function to an alcohol (ß-ketoacyl-ACP reductase), dehydration to form an enoyl-ACP (ß-hydroxyacyl-ACP dehydrase), and finally reduction of the enoyl-ACP to form the elongated saturated acyl-ACP (enoyl-ACP reductase). ß-ketoacyl-ACP synthase I (KAS I), is primarily responsible for elongation up to palmitoyl-ACP (C16:0), whereas ß-ketoacyl-ACP synthase II (KAS II) is predominantly responsible for the final elongation to stearoyl-ACP (C18:0).

Genes encoding peptide components of ß-ketoacyl-ACP synthases I and II have been cloned from a number of higher plant species, including castor (*Ricinus communis*) and *Brassica* species (USPN 5,510,255). KAS I activity was associated with a single synthase protein factor having an approximate molecular weight of 50 kD (synthase factor B) and KAS II activity was associated with a combination of two synthase protein factors, the 50 kD synthase factor B and a 46 kd protein designated synthase factor A. Cloning and sequence of a plant gene encoding a KAS III protein has been reported by Tai and Jaworski (*Plant Physiol.* (1993) *103*:1361-1367).

The end products of plant fatty acid synthetase activities are usually 16- and 18-carbon fatty acids. There are, however, several plant families that store large amounts of 8- to 14-carbon (medium-chain) fatty acids in their oilseeds. Recent studies with *Umbellularia californica* (California bay), a plant that produces seed oil rich in lauric acid (12:0), have demonstrated the existence of a medium-chain-specific isozyme of acyl-ACP thioesterase

in the seed plastids. Subsequent purification of the 12:0-ACP thioesterase from *Umbellularia californica* led to the cloning of a thioesterase cDNA which was expressed in seeds of *Arabidopsis* and *Brassica* resulting in a substantial accumulation of lauric acid in the triglyceride pools of these transgenic seeds (USPN 5,512,482). These results and subsequent studies with medium-chain thioesterases from other plant species have confirmed the chain-length-determining role of acyl-ACP thioesterases during de novo fatty acid biosynthesis (T. Voelker (1996) *Genetic Engineering*, Ed. J. K. Setlow, Vol. 18, pgs. 111-133).

DESCRIPTION OF THE FIGURES

Figure 1. DNA and translated amino acid sequence of *Cuphea hookeriana* KAS factor B clone chKAS B-2 are provided.

Figure 2. DNA and translated amino acid sequence of *Cuphea hookeriana* KAS factor B clone chKAS B-31-7 are provided.

Figure 3. DNA and translated amino acid sequence of *Cuphea hookeriana* KAS factor A clone chKAS A-2-7 are provided.

Figure 4. DNA and translated amino acid sequence of *Cuphea hookeriana* KAS factor A clone chKAS A-1-6 are provided.

Figure 5. DNA and translated amino acid sequence of *Cuphea pullcherrima* KAS factor B clone cpuKAS B/7-8 are provided.

Figure 6. DNA and translated amino acid sequence of *Cuphea pullcherrima* KAS factor B clone cpuKAS B/8-7A are provided.

Figure 7. DNA and translated amino acid sequence of *Cuphea pullcherrima* KAS factor A clone cpuKAS A/p7-6A are provided.

Figure 8. Preliminary DNA sequence of *Cuphea pullcherrima* KAS factor A clone cpuKAS A/p8-9A is provided.

Figure 9. DNA and translated amino acid sequence of *Cuphea hookeriana* KASIII clone chKASIII-27 are provided.

Figure 10. The activity profile for purified cpuKAS B/8-7A using various acyl-ACP substrates is provided.

Figure 11. The activity profile for purified chKAS A-2-7 and chKAS A-1-6 using various acyl-ACP substrates is provided.

Figure 12. The activity profile for purified castor KAS factor A using various acyl-ACP substrates is provided.

Figure 13. The activity profile for purified castor KAS factor B using various acyl-ACP substrates is provided.

Figure 14. A graph showing the number of plants arranged according to C8:0 content for transgenic plants containing CpFatB1 versus transgenic plants containing CpFatB1 + chKAS A-2-7 is provided.

Figure 15. Graphs showing the %C10/%C8 ratios in transgenic plants containing ChFatB2 (4804-22-357) and in plants resulting from crosses between 4804-22-357 and 5401-9 (chKAS A-2-7 plants) are provided.

Figure 16. Graphs showing the %C10 + %C8 contents in transgenic plants containing ChFatB2 (4804-22-357) and in plants resulting from crosses between 4804-22-357 and 5401-9 (chKAS A-2-7 plants) are provided.

Figure 17. Graphs showing the %C10/%C8 ratios in transgenic plants containing ChFatB2 (4804-22-357) and in plants resulting from crosses between 4804-22-357 and 5413-17 (chKAS A-2-7 + CpFatB1 plants) are provided.

Figure 18. Graphs showing the %C10 + %C8 contents in transgenic plants containing ChFatB2 (4804-22-357) and in

plants resulting from crosses between 4804-22-357 and 5413-17 (chKAS A-2-7 + CpFatB1 plants) are provided.

Figure 19. Graphs showing the %C12:0 in transgenic plants containing Uc FatB1 (LA86DH186) and in plants resulting from crosses with wild type (X WT) and with lines expressing Ch KAS A-2-7.

Figure 20. Graph showing the relative proportions of C12:0 and C14:0 fatty acids in the seeds of transgenic plants containing Uc FatB1 (LA86DH186) and in plants resulting from crosses with wild type (X WT) and with lines expressing Ch KAS A-2-7.

Figure 21. Graphs showing the %C18:0 in transgenic plants containing Garm FatB1 (5266) and in seeds of plants resulting from crosses with wild type (X WT) and with lines expressing Ch KAS A-2-7.

Figure 22. The activity profile of Ch KAS A in protein extracts from transgenic plants containing Ch KAS A-2-7. Extracts were preptreated with the indicated concentrations of cerulenin.

SUMMARY OF THE INVENTION

By this invention, compositions and methods of use related to ß-ketoacyl-ACP synthase (KAS) are provided. Also of interest are methods and compositions of amino acid and nucleic acid sequences related to biologically active plant synthase(s).

In particular, genes encoding KAS protein factors A and B from *Cuphea* species are provided. The KAS genes are of interest for use in a variety of applications, and may be

used to provide synthase I and/or synthase II activities in transformed host cells, including bacterial cells, such as *E. coli*, and plant cells. Synthase activities are distinguished by the preferential activity towards longer and shorter acyl-ACPs as well as by the sensitivity towards the KAS specific inhibitor, cerulenin. Synthase protein preparations having preferential activity towards medium chain length acyl-ACPs are synthase I-type or KAS I. The KAS I class is sensitive to inhibition by cerulenin at concentrations as low as 1µM. Synthases having preferential activity towards longer chain length acyl-ACPs are synthase II-type or KAS II. The KAS enzymes of the II-type are also sensitive to cerulenin, but at higher concentrations (50µM). Synthase III-type enzymes have preferential activity towards short chain length acyl-ACPs and are insensitive to cerulenin inhibition.

Nucleic acid sequences encoding a synthase protein may be employed in nucleic acid constructs to modulate the amount of synthase activity present in the host cell, especially the relative amounts of synthase I-type, synthase II-type and synthase III-type activity when the host cell is a plant host cell. In addition, nucleic acid constructs may be designed to decrease expression of endogenous synthase in a plant cell as well. One example is the use of an anti-sense synthase sequence under the control of a promoter capable of expression in at least those plant cells which normally produce the enzyme.

Of particular interest in the present invention is the coordinate expression of a synthase protein with the

expression of thioesterase proteins. For example, coordinated expression of synthase factor A and a medium-chain thioesterase provides a method for increasing the level of medium-chain fatty acids which may be harvested from transgenic plant seeds. Furthermore, coordinated expression of a synthase factor A gene with plant medium-chain thioesterase proteins also provides a method by which the ratios of various medium-chain fatty acids produced in a transgenic plant may be modified. For example, by expression of a synthase factor A, it is possible to increase the ratio of C10/C8 fatty acids which are produced in plant seed oils as the result of expression of a thioesterase having activity on C8 and C10 fatty acids.

DETAILED DESCRIPTION OF THE INVENTION

A plant synthase factor protein of this invention includes a sequence of amino acids or polypeptide which is required for catalyzation of a condensation reaction between an acyl-ACP having a chain length of C_2 to C_{16} and malonyl-ACP in a plant host cell. A particular plant synthase factor protein may be capable of catalyzing a synthase reaction in a plant host cell (for example as a monomer or homodimer) or may be one component of a multiple peptide enzyme which is capable of catalyzing a synthase reaction in a plant host cell, i.e. one peptide of a heterodimer.

Synthase I (KAS I) demonstrates preferential activity towards acyl-ACPs having shorter carbon chains, C_2-C_{14} and is sensitive to inhibition by cerulenin at concentrations of $1\mu M$. Synthase II (KAS II) demonstrates preferential

activity towards acyl-ACPs having longer carbon chains, C_{14}-C_{16}, and is inhibited by concentrations of cerulenin (50μM). Synthase III demonstrates preferential activity towards acyl-CoAs having very short carbon chains, C_2 to C_6, and is insensitive to inhibition by cerulenin.

Synthase factors A and B, and synthase III proteins obtained from medium-chain fatty acid producing plant species of the genus *Cuphea* are described herein. As described in the following Examples, synthase A from *C. hookeriana* is naturally expressed at a high level and only in the seeds. *C. hookeriana* synthase B is expressed at low levels in all tissues examined. Expression of synthase A and synthase B factors in *E. coli* and purification of the resulting proteins is employed to determine activity of the various synthase factors. Results of these analyses indicate that synthase factor A from *Cuphea hookeriana* has the greatest activity on 6:0-ACP substrates, whereas synthase factor B from *Cuphea pullcherrima* has greatest activity on 14:0-ACP. Similar studies with synthase factors A and B from castor demonstrate similar activity profiles between the factor B synthase proteins from *Cuphea* and castor. The synthase A clone from castor, however, demonstrates a preference for 14:0-ACP substrate.

Expression of a *Cuphea hookeriana* KAS A protein in transgenic plant seeds which normally do not produce medium-chain fatty acids does not result in any detectable modification of the fatty acid types and contents produced in such seeds. However, when *Cuphea hookeriana* KAS A protein is expressed in conjunction with expression of a

medium-chain acyl-ACP thioesterase capable of providing for production of C8 and C10 fatty acids in plant seed oils, increases in the levels of medium-chain fatty acids over the levels obtainable by expression of the medium-chain thioesterase alone are observed. In addition, where significant amounts of C8 and C10 fatty acids are produced as the result of medium-chain thioesterase expression, co-expression of a *Cuphea* KAS A protein also results in an alteration of the proportion of the C8 and C10 fatty acids that are obtained. For example, an increased proportion of C10 fatty acids may be obtained by co-expression of *Cuphea hookeriana* ChFatB2 thioesterase and a chKAS A synthase factor proteins.

Furthermore, when *Cuphea hookeriana* KAS A protein is expressed in conjunction with expression of a medium-chain acyl-ACP thioesterase capable of providing for production of C12 fatty acids in plant seed oils, increases in the levels of medium-chain fatty acids over the levels obtainable by expression of the medium-chain thioesterase alone are also observed. In addition, where significant amounts of C12 and C14 fatty acids are produced as the result of medium-chain thioesterase expression, co-expression of a *Cuphea* KAS A protein also results in an alteration of the proportion of the C12 and C14 fatty acids that are obtained. For example, an increased proportion of C12 fatty acids may be obtained by co-expression of *Uc* FatB1 thioesterase and a chKAS A synthase factor proteins.

However, when *Cuphea hookeriana* KAS A protein is expressed in conjunction with the expression of a long-chain

acyl-ACP thioesterase capable of providing for production of C18 and C18:1 fatty acids in plant seed oils, no effect on the production of long chain fatty acids was observed. Furthermore, when plants transformed to express a long chain acyl-ACP thioesterase from mangosteen (*Garm*FatA1, U.S. Patent Application No. 08/440,845), which preferentially hydrolyzes C18:0 and C18:1 fatty acyl-ACPs, are crossed with nontransformed control plants, a significant reduction in the levels of C18:0 is obtained. Similar reductions are also observed in the levels of C18:0 in the seeds of plants resulting from crosses between plants transformed to express the *Garm*FatA1 and plants expressing the *Cuphea hookeriana* KAS A protein.

Thus, the instant invention provides methods of increasing and/or altering the medium-chain fatty acid compositions in transgenic plant seed oils by co-expression of medium-chain acyl-ACP thioesterases with synthase factor proteins. Furthermore, various combinations of synthase factors and medium-chain thioesterases may be achieved depending upon the particular fatty acids desired. For example, for increased production of C14 fatty acids, synthase protein factors may be expressed in combination with a C14 thioesterase, for example from *Cuphea palustris* or nutmeg may be employed (WO 96/23892). In addition, thioesterase expression may be combined with a number of different synthase factor proteins for additional effects on medium-chain fatty acid composition.

Synthases of use in the present invention include modified amino acid sequences, such as sequences which have

been mutated, truncated, increased and the like, as well as such sequences which are partially or wholly artificially synthesized. The synthase protein encoding sequences provided herein may be employed in probes for further screening or used in genetic engineering constructs for transcription or transcription and translation in host cells, especially plant host cells. One skilled in the art will readily recognize that antibody preparations, nucleic acid probes (DNA and RNA) and the like may be prepared and used to screen and recover synthases and/or synthase nucleic acid sequences from other sources. Typically, a homologously related nucleic acid sequence will show at least about 60% homology, and more preferably at least about 70% homology, between the *R. communis* synthase and the given plant synthase of interest, excluding any deletions which may be present. Homology is determined upon comparison of sequence information, nucleic acid or amino acid, or through hybridization reactions.

Recombinant constructs containing a nucleic acid sequence encoding a synthase protein factor or nucleic acid sequences encoding a synthase protein factor and a medium-chain acyl-ACP thioesterase may be prepared by methods well known in the art. Constructs may be designed to produce synthase in either prokaryotic or eukaryotic cells. The increased expression of a synthase in a plant cell, particularly in conjunction with expression of medium-chain thioesterases, or decreasing the amount of endogenous synthase observed in plant cells are of special interest.

Synthase protein factors may be used, alone or in combination, to catalyze the elongating condensation reactions of fatty acid synthesis depending upon the desired result. For example, rate influencing synthase activity may reside in synthase I-type, synthase II-type, synthase III-type or in a combination of these enzymes. Furthermore, synthase activities may rely on a combination of the various synthase factors described herein.

Constructs which contain elements to provide the transcription and translation of a nucleic acid sequence of interest in a host cell are "expression cassettes". Depending upon the host, the regulatory regions will vary, including regions from structural genes from viruses, plasmid or chromosomal genes, or the like. For expression in prokaryotic or eukaryotic microorganisms, particularly unicellular hosts, a wide variety of constitutive or regulatable promoters may be employed. Among transcriptional initiation regions which have been described are regions from bacterial and yeast hosts, such as *E. coli, B. subtilis, Saccharomyces cerevisiae*, including genes such as ß-galactosidase, T7 polymerase, trp-lac (tac), trp E and the like.

An expression cassette for expression of synthase in a plant cell will include, in the 5' to 3' direction of transcription, a transcription and translation initiation control regulatory region (also known as a "promoter") functional in a plant cell, a nucleic acid sequence encoding a synthase, and a transcription termination region. Numerous transcription initiation regions are available

which provide for a wide variety of constitutive or regulatable, e.g., inducible, transcription of the desaturase structural gene. Among transcriptional initiation regions used for plants are such regions associated with cauliflower mosaic viruses (35S, 19S), and structural genes such as for nopaline synthase or mannopine synthase or napin and ACP promoters, etc. The transcription/ translation initiation regions corresponding to such structural genes are found immediately 5' upstream to the respective start codons. Thus, depending upon the intended use, different promoters may be desired.

Of special interest in this invention are the use of promoters which are capable of preferentially expressing the synthase in seed tissue, in particular, at early stages of seed oil formation. Examples of such seed-specific promoters include the region immediately 5' upstream of a napin or seed ACP genes such as described in USPN 5,420,034, desaturase genes such as described in Thompson *et al* (*Proc. Nat. Acad. Sci.* (1991) *88:2578-2582)*, or a Bce-4 gene such as described in USPN 5,530,194. Alternatively, the use of the 5' regulatory region associated with the plant synthase structural gene, i.e., the region immediately 5' upstream to a plant synthase structural gene and/or the transcription termination regions found immediately 3' downstream to the plant synthase structural gene, may often be desired. In general, promoters will be selected based upon their expression profile which may change given the particular application.

In addition, one may choose to provide for the transcription or transcription and translation of one or more other sequences of interest in concert with the expression or anti-sense of the synthase sequence, particularly medium-chain plant thioesterases such as described in USPN 5,512,482, to affect alterations in the amounts and/or composition of plant oils.

When one wishes to provide a plant transformed for the combined effect of more than one nucleic acid sequence of interest, a separate nucleic acid construct may be provided for each or the constructs may both be present on the same plant transformation construct. The constructs may be introduced into the host cells by the same or different methods, including the introduction of such a trait by crossing transgenic plants via traditional plant breeding methods, so long as the resulting product is a plant having both characteristics integrated into its genome.

Normally, included with the DNA construct will be a structural gene having the necessary regulatory regions for expression in a host and providing for selection of transformed cells. The gene may provide for resistance to a cytotoxic agent, e.g. antibiotic, heavy metal, toxin, etc., complementation providing prototrophy to an auxotrophic host, viral immunity or the like. Depending upon the number of different host species into which the expression construct or components thereof are introduced, one or more markers may be employed, where different conditions for selection are used for the different hosts.

The manner in which the DNA construct is introduced into the plant host is not critical to this invention. Any method which provides for efficient transformation may be employed. Various methods for plant cell transformation include the use of Ti- or Ri-plasmids, microinjection, electroporation, liposome fusion, DNA bombardment or the like. In many instances, it will be desirable to have the construct bordered on one or both sides by T-DNA, particularly having the left and right borders, more particularly the right border. This is particularly useful when the construct uses *A. tumefaciens* or *A. rhizogenes* as a mode for transformation, although the T-DNA borders may find use with other modes of transformation.

The expression constructs may be employed with a wide variety of plant life, particularly plant life involved in the production of vegetable oils. These plants include, but are not limited to rapeseed, peanut, sunflower, safflower, cotton, soybean, corn and oilseed palm.

For transformation of plant cells using *Agrobacterium*, explants may be combined and incubated with the transformed *Agrobacterium* for sufficient time for transformation, the bacteria killed, and the plant cells cultured in an appropriate selective medium. Once callus forms, shoot formation can be encouraged by employing the appropriate plant hormones in accordance with known methods and the shoots transferred to rooting medium for regeneration of plants. The plants may then be grown to seed and the seed used to establish repetitive generations and for isolation of vegetable oils.

The invention now being generally described, it will be more readily understood by reference to the following examples which are included for purposes of illustration only and are not intended to limit the present invention.

EXAMPLES

Example 1 Cuphea KAS Factor A and B Gene Cloning

Total RNA isolated from developing seeds of *Cuphea hookeriana* and *Cuphea pullcherrima* was used for cDNA synthesis in commercial 1-based cloning vectors. For cloning each type of KAS gene, approximately 400,000-500,000 unamplified recombinant phage were plated and the plaques transferred to nitrocellulose. For KAS factor B cloning from *C. hookeriana*, a mixed probe containing *Brassica napus* KAS factor B and *Ricinus communis* (Castor) KAS factor B radiolabeled cDNA's was used. Similarly, a mixed probe containing *Brassica napus* KAS factor A and *Ricinus communis* KAS factor A cDNA clones was used to obtain *C. hookeriana* KAS factor A genes. For KASIII, a spinach KASIII cDNA clone obtained from Dr. Jan Jaworski was radiolabeled and used as a probe to isolate a KASIII clone from *C. hookeriana*. For KAS B and KAS A cloning from *C. pullcherrima*, *C. hookeriana* KAS B and KAS A genes chKAS B-2 and chKAS A-2-7 (see below) were radiolabeled and used as probes.

DNA sequence and translated amino acid sequence for *Cuphea* KAS clones are provided in Figures 1-9. *Cuphea hookeriana* KAS factor B clones chKAS B-2 and chKAS B-31-7

are provided in Figures 1 and 2. Neither of the clones is full length. *Cuphea hookeriana* KAS Factor A clones chKAS A-2-7 and chKAS A-1-6 are provided in Figures 3 and 4. chKAS A-2-7 contains the entire encoding sequence for the KAS factor protein. Based on comparison with other plant synthase proteins, the transit peptide is believed to be represented in the amino acids encoded by nucleotides 125-466. chKAS A-1-6 is not a full length clone although some transit peptide encoding sequence is present. Nucleotides 1-180 represent transit peptide encoding sequence, and the mature protein encoding sequence is believed to begin at nucleotide 181.

Cuphea pullcherrima KAS factor B clones cpuKAS B/7-8 and cpuKAS B/8-7A are provided in Figures 5 and 6. Both of the clones contain the entire encoding sequences for the KAS factor B proteins. The first 35 amino acids of cpuKAS B/7-8 are believed to represent the transit peptide, with the mature protein encoding sequence beginning at nucleotide 233. The first 39 amino acids of cpuKAS B/8-7A are believed to represent the transit peptide, with the mature protein encoding sequence beginning at nucleotide 209. *Cuphea pullcherrima* KAS factor A clones cpuKAS A/p7-6A and cpuKAS A-p8-9A are provided in Figures 7 and 8. Both of the clones contain the entire encoding sequences for the KAS factor A proteins. Translated amino acid sequence of cpuKAS A/p7-6A is provided. The mature protein is believed to begin at the lysine residue encoded 595-597, and the first 126 amino acids are believed to represent the transit peptide. The DNA sequence of KAS A clone cpuKAS A-p8-9A is preliminary.

Further analysis will be conducted to determine final DNA sequence and reveal the amino acid sequence encoded by this gene.

DNA and translated amino acid sequence of *Cuphea hookeriana* KASIII clone chKASIII-27 is provided in Figure 9. The encoding sequence from nucleotides 37-144 of chKASIII-27 are believed to encode a transit peptide, and the presumed mature protein encoding sequence is from nucleotides 145-1233.

Deduced amino acid sequence of the *C. hookeriana* KAS factor B and KAS factor A cDNA's reveals strong homology to the *Brassica napus* and *Ricinus communis* clones previously reported. The *C. hookeriana* KAS factor B clone is more homologous to the *Ricinus* and *Brassica* KAS factor B clones (94% and 91% respectively) than it is to the *Ricinus* and *Brassica* KAS factor A clones (60% for both). Furthermore, the *C. hookeriana* KAS factor A clone is more homologous to the *Ricinus* and *Brassica* KAS factor A clones (85% and 82% respectively) than it is the *Ricinus* and *Brassica* KAS factor B clone (60% for both). The *C. hookeriana* KAS factor B cDNAs designated as chKAS B-2 and chKAS B-31-7 are 96% identical within the mature portion of the polypeptide. Similarly, the deduced amino acid sequence of the mature protein regions of the *C. hookeriana* KAS factor A clones chKAS A-2-7 and chKAS A-1-6 are 96% identical. The *C. pullcherrima* KAS clones also demonstrate homology to the *R. communis* and *Brassica napus* KAS clones. The mature protein portion of all of the KAS factor A family members in the different *Cuphea* species are 95% identical. Similarly the

mature protein portion of the KAS factor B genes in *Cuphea* are also 95-97% identical with each other. However there is only approximately 60% sequence identity between KAS factor B and KAS factor A clones either within the same or different species of *Cuphea*.

<u>Example 2</u> Levels and Patterns of Expression

To examine tissue specificity of KAS expression in *Cuphea hookeriana*, Northern blot analysis was conducted using total RNA isolated from seed, root, leaf and flower tissue. Two separate but identical blots were hybridized with either chKAS B-31-7 or chKAS A-2-7 coding region probes. The data from this RNA blot analysis indicate that KAS B is expressed at a similar level in all tissues examined, whereas KAS A expression is detected only in the seed. These results also demonstrate a different level of expression for each of the synthases. KAS A is an abundant message, whereas KAS B is expressed at low levels. Furthermore, even under highly stringent hybridization conditions (65_C, 0.1 X SSC, 0.5% SDS), the KAS A probe hybridizes equally well with two seed transcripts of 2.3 and 1.9 kb. The larger hybridizing band is likely the transcript of the KAS A-2-7 gene since the size of its cDNA is 2046bp, and the number of clones obtained from cDNA screening corresponds well with the apparent mobility of the mRNA and its abundance on the blot.

<u>Example 3</u> Expression of Plant KAS Genes in E.coli

DNA fragments encoding the mature polypeptide of the *Cuphea hookeriana* KAS A cDNAs and the *Cuphea pullcherrima* KAS B cDNAs were obtained by PCR and cloned into a QIAexpress expression vector (Qiagene). Experimental conditions for maximum level of expression were determined for all of these clones and the parameters for highest level of soluble fraction were identified. Cells are grown in ECLB media containing 1M sorbitol and 2.5 mM betaine overnight and subcultured as a 1:4 dilution in the same medium. Cells are then grown for 2 hours (to approximately .6-.8 O.D.) and induced with 0.4 mM IPTG and allowed to grow for 5 more hours.

Enzyme activity of the affinity purified recombinant enzymes obtained from over-expression of the chKAS A-2-7 and cpuKAS B/8-7A clones was measured using a wide range of acyl-ACP substrates (6:0- to 16:1-ACP). The activity profile for cpuKAS B/8-7A is provided in Fig.10. The data demonstrate that the enzyme is active with all acyl-ACP substrates examined, although activity on 6:0 to 14:0-ACP substrates is substantially greater than the activity on 16:0 and 16:1 substrates.

The activity profile of the *C. hookeriana* KAS A clones chKAS A-2-7 and chKAS A-1-6 is provided in Figure 11. The *C. hookeriana* KAS A clones are most active with C:6, and have the least activity with C:16:0 substrates. However, the activity of this clone on even the preferred C6:0 substrate

is 50 fold lower than the activity of the *C. pullcherrima* KAS B clones.

A fragment containing the mature protein encoding portion of a *R. communis* KAS factor A clone was also cloned into a QIAexpress expression vector, expressed in *E. coli* and the enzyme affinity purified as described above. The activity profile for castor KAS A is provided in Figure 12. Highest activity is observed with C14:0 substrates, although some activity is also seen with C6:0 and C16:1. In comparison, the activity profile obtained from purified *R. communis* KAS factor B also using the QIAexpress expression system is provided in Figure 13. The KAS B clone demonstrates substantially higher levels of activity (10 fold and higher) than the *R. communis* KAS A clone. The preference of the KAS factor B for 6:0- to 14:0-ACP substrates is consistent with the previous observations that this protein provides KAS I activity.

<u>Example 4</u> KAS and TE Expression in Transgenic Seed

Both the CpFatB1 (*C. hookeriana* thioesterase cDNA; Dehesh *et al.* (1996) *Plant Physiol. 110*:203-210) and the chKAS A-2-7 were PCR amplified, sequenced, and cloned into a napin expression cassette. The napin/cp FatB1 and the napin/KAS A-2-7 fusions were ligated separately into the binary vector pCGN1558 (McBride and Summerfelt (*Pl.Mol.Biol.* (1990) *14*:269-276) and transformed into *A. tumefaciens*, EHA101. The resulting CpFatB1 binary construct is pCGN5400 and the chKAS A-2-7 construct is pCGN5401. *Agrobacterium* mediated transformation of a *Brassica napus* canola variety

was carried out as described by Radke *et al.* (*Theor. Appl. Genet.* (1988) *75*:685-694; *Plant Cell Reports* (1992) *11*:499-505). Several transgenic events were produced for each of the pCGN5400 and pCGN5401 constructs.

A double gene construct containing a napin/cpFatB1 expression construct in combination with a napin/chKAS A-2-7 expression construct was also assembled, ligated into a binary vector and used for co-cultivation of a canola *Brassica* variety. The binary construct containing the chFatB1 and chKAS A-2-7 expression constructs is pCGN5413.

Fatty acid analysis of 26 transgenic lines containing chKAS A-2-7 (5401 lines) showed no significant changes in the oil content or profile as compared to similar analyses of wild type canola seeds of the transformed variety.

Fatty acid analysis of 36 transgenic lines containing cpFatB1 (5400 lines) showed increased levels of C:8 and C:10 in transgenic seeds. The highest level of C:8 observed in a pool seed sample was 4.2 mol%. The C:10 levels were between 30 and 35% of the C:8 content. Fatty acid analysis of 25 transgenic lines containing the TE/KAS A tandem (5413 lines) demonstrated an overall increase in both C:8 and C:10 levels relative to those observed with TE containing lines (5400) alone. In lines containing the cpFatB1 construct alone, the average level of C:8 average were 1.5 mol%, whereas the C:8 average levels in TE/KAS A tandem containing lines was 2.37 mol%. The ratio of C:8 to C:10 remained constant in both populations. The number of transgenic events relative to the C:8 content are presented in Figure 14. These data show that the transgenic events with tandem TE/KAS A construct

yield more lines with higher levels of C:8 than those events with single TE construct. For example, several lines containing nearly 7 mole% C8 were obtained with the TE/KAS A pCGN5413 construct, whereas the highest C8 containing line from the pCGN5400 TE alone transformation contained 4.2 mole% C8.

Half seed analysis of the T3 generation of transgenic canola plants expressing a ChFatB2 (*C. hookeriana* thioesterase; Dehesh *et al.* (1996) *The Plant Journal 9*:167-172) indicate that these plant can accumulate up to 22 weight% (33 mol%) of 8:0 and 10:0 fatty acids (4804-22-357). Segregation analysis shows that these transformants contain two loci and that they are now homozygous. Selected plants grown from these half seeds were transferred into the greenhouse and later crossed with T1 transformants that had been transformed with either *Cuphea hookeriana* KAS A (5401) alone or KAS A/CpFatB1 double constructs (5413).

Fatty acid analysis of several events resulting from the crosses between transgenic lines containing ChFatB2 (4804-22-357) and chKAS A-2-7 (5401-9), reveal an increase in the ratio of C:10/C:8 levels (Figure 15). This C:10/C:8 ratio in nearly all of the transgenic events containing ChFatB2 TE alone fluctuates between 3 and 6, whereas in the F1 generation of transgenic containing both the TE and the KAS A-2-7, the ratio can be as high as 22. This increase in C:10 levels is accompanied by an increase in the total C:8 and C:10 content (Figure 16). The sum of the C:8 and C:10 fatty acids in the heterozygous F1 lines is as high as those in the homozygous parent line (4804-22-357), whereas the

heterozygous lines usually contain substantially less C:8 and C:10 than the homozygous lines.

Similar results were observed in F1 generation seeds resulting from crosses performed between 4804-22-357 (ChFatB2) and the 5413-17 event (CpFatB1 and chKAS A-2-7 tandem). Levels of C:8 and C:10 in the 5413-17 line were 6.3 and 2.8 mol% respectively. Data presented in Figure 17 show that there is shift towards C:10 fatty acids as was observed with the 4804-22-357 (ChFatB2) x 5401-9 (chKAS A-2-7) crosses. Furthermore, Figure 18 indicates the presence of two separate populations of heterozygotes. Those containing approximately 9-11 weight percent C:10 + C:8 are believed to represent offspring containing a single copy of the ChFatB1 TE gene and no copies of the CpFatB1 and chKAS A genes from 5413. Those plants containing approximately 15-20 weight percent C:10 + C:8 are believed to represent the heterozygotes containing a single ChFatB1 TE gene as well as the CpFatB1 and chKAS A genes from 5413. Thus, the level of the C:10 + C:8 fatty acids does not decrease to 50% of that detected in parent lines when a copy of the ChKAS A gene is present.

To further characterize the chain length specificity of the *Cuphea hookeriana* KAS A enzyme, crosses between transgenic *Brassica napus* lines containing a California Bay (*Umbellularia californica*) 12:0 specific thioesterase, Uc FatB1 (USPN 5,344,771) and chKAS A-2-7 (5401-9) were made. Half seed analysis of transgenic plants containing Uc fatB1 have previuosly indicated that these plants can accumulate up to 52 mol% C12:0 in the seed oil of homozygous dihaploid

lines (LA86DH186). Crosses between the line LA86DH186 and untransformed control *Brassica* demonstrated a decrease in the C12:0 levels.

However, crosses between LA86DH186 and the 5401-9 hemizygous line led to an accumulation of up to 57 mol% C12:0 in the seed oil of F1 progeny (Figure 19). Interestingly, in crosses with LA86DH186 x untransformed control line and LA86DH186 x 5401-9, levels of C14:0 in the seeds of the F1 progeny decreased to 50% of the levels obtained in homozygous LA86DH186 lines (Figure 20). Furthermore, increases in the proportion of C12:0 fatty acid resulted in a substantial decline in the proportions of all the long-chain fatty acyl groups (C16:0, C18:0, C18:2, and C18:3). These results indicate that the ChKAS A-2-7 is an enzyme with substrate specificity ranging from C6:0 to C10:0-ACP, and that its over-expression ultimately reduces the longer chain acyl-ACP pools.

Further evidence is obtained in support of the chain length specificity of the ChKAS A-2-7 in crosses of the 5401-9 line with a transgenic line (5266) expressing an 18:1/18:0 TE from *Garcinia mangostana* (GarmFatA1, US patent application No. 08/440,845). Transgenic *Brassica* line 5266 has been shown to accumulate up to 24 mol% C18:0 in the seed oil of homozygous lines (Figure 21). However, in the seed oil of F1 progeny of crosses between 5266 and 5401-9 levels of C18:0 were reduced to approximately 12 mol%. Furthermore, levels of C16:0 generated from these crosses was similar to the levels obtained from the seed oil of nontransgenic control plants.

Example 5 ***In vitro* Analysis of Plant KAS Enzymes**

Seed extracts were prepared from developing seeds of nontransgenic controls or transgenic *Brassica* expressing chKAS A-2-7 as described in Slabaugh *et al.*(*Plant Journal,* 1998 in press) and Leonard *et al.* (Plant Journal, 1998, in press). *In vitro* fatty acid synthesis assays were performed as described by Post-Beittenmiller (*J. Biol. Chem.* (1991), 266:1858-1865). Extracts were concentrated by ammonium sulfate precipitation and desalting using P-6 columns (Bio-Rad, Hercules, CA). Reactions (65µl) contained 0.1M Tris/HCl (pH 8.0), 1 mM dithiothreitol, 25 mM recombinant spinach ACP1, 1 mM NADH, 2 mM NADPH, 50 µM malonyl-CoA, 10 µM [1-^{14}C]acetyl-CoA (50 mCi/mmol), 1mg/ml BSA, and 0.25 mg/ml seed protein. Selected seed extracts were preincubated with cerulenin at 23°C for 10 min. Reaction products were separated on an 18% acrlamide gel containing 2.25M urea, electroblotted onto to nitrocellulose and quntitated by phosporimaging using Image QuaNT software (Molecular Dynamics, Sunnyvale, CA). Authentic acyl-ACPs were run in parallel, immunoblotted and finally detected by anti-ACP serum to confirm fatty acid chain lengths.

The results (Figure 22) indicate that the fatty acid synthesis capabilities of transgenic *Brasica* (5401-9) seed extracts was greater than that obtained from in the nontransgenic controls as measured by the relative abundance of C8:0- and C10:0-ACP at all time points tested. In addition, pretreatment of the extracts with cerulenin, markedly reduced the synthesis of longer chain fatty acids in both the transgenic and nontransgenic control seed

extracts. However, the extension of the spinach-ACP was much less inhibited in the seed extracts from the transgenic lines than in the seed extracts of nontransgenic control *Brassica*.

These data further support that Ch KAS A-2-7 is a condensing enzyme active on medium chain acyl-ACPs, and that expression of this enzyme in plants results in enlarged substrate pools to be hydrolyzed by medium-chain specific thioesterases. Furthermore, these data suggest that chKAS A-2-7 also is a cerulenin-resistant condensing enzyme.

All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claim.

MISSING UPON TIME OF PUBLICATION

13. The construct of Claim 5 wherein said encoding sequence is cpuKAS A/p8-9A.

14. The construct of Claim 5 wherein said encoding sequence is chKASIII-27.

15. An improved method for producing medium-chain fatty acids in transgenic plant seeds by expression of a plant medium-chain thioesterase protein heterologous to said transgenic plant,

the improvement comprising expression of a plant synthase factor protein heterologous to said transgenic plant in conjunction with expression of said plant medium-chain thioesterase, whereby the percentage of medium-chain fatty acids produced in seeds expressing both a plant synthase factor protein and a plant medium-chain thioesterase protein is increased as compared to the percentage of medium-chain fatty acids produced in seeds expressing only said plant medium-chain thioesterase protein.

16. The method of Claim 15 wherein said medium-chain thioesterase protein is a ChFatB2 protein.

17. The method of Claim 15 wherein said medium-chain thioesterase protein is a CpFatB1 protein.

18. The method of Claim 15 wherein said medium-chain thioesterase protein is a C12 preferring thioesterase from California bay.

19. The method of Claim 15 wherein said plant synthase factor protein is expressed from a construct according to Claim 1.

20. The method of Claim 19 wherein said synthase factor A protein is from a *Cuphea* species.

21. The method of Claim 20 wherein said *Cuphea* species is *C. hookeriana* or *C. pullcherrima*.

22. A method of altering the medium-chain fatty acid composition in plant seeds expressing a heterologous plant medium-chain preferring thioesterase, wherein said method comprises

providing for expression of a plant synthase factor protein heterologous to said transgenic plant in conjunction with expression of a plant medium-chain thioesterase protein heterologous to said transgenic plant, whereby the composition of medium-chain fatty acids produced in said seeds is modified as compared to the composition of medium-chain fatty acids produced in seeds expressing said plant medium-chain thioesterase protein in the absence of expression of said plant synthase factor protein.

23. The method of Claim 22 wherein said medium-chain thioesterase protein is a ChFatB2 protein.

24. The method of Claim 22 wherein said medium-chain thioesterase protein is a CpFatB1 protein.

25. The method of Claim 22 wherein said medium-chain thioesterase protein is a C12 preferring thioesterase from California bay.

26. The method of Claim 22 wherein said plant synthase factor protein is expressed from a construct according to Claim 1.

27. The method of Claim 26 wherein said synthase factor A protein is from a *Cuphea* species.

28. The method of Claim 27 wherein said *Cuphea* species is *C. hookeriana* or *C. pullcherrima*.

29. The method of Claim 22 wherein said fatty acid composition is enriched for C10 fatty acids.

30. The method of Claim 22 wherein said fatty acid composition is enriched for C12 fatty acids.

31. The method of Claim 22 wherein said fatty acid composition is enriched for at least one medium chain fatty acid and at least one other medium chain fatty acid is decreased.

32. The method of Claim 31 wherein said enriched fatty acid is C12 and said decreased fatty acid is C14.

```
AGC TCC ACC GCG GTG GCG GCC GCT CTA GAA CTA GTG GAT CCC CCG GGC    48
Ser Ser Thr Ala Val Ala Ala Ala Leu Glu Leu Val Asp Pro Pro Gly

TGC AGG AAT TCG GCA CGA GCC GAT CTC GGT GCC GAC CGC CTC TCC AAG    96
Cys Arg Asn Ser Ala Arg Ala Asp Leu Gly Ala Asp Arg Leu Ser Lys

ATC GAC AAG GAG AGA GCC GGA GTG CTG GTC GGA ACA GGA ATG GGT GGT   144
Ile Asp Lys Glu Arg Ala Gly Val Leu Val Gly Thr Gly Met Gly Gly

CTG ACT GTC TTC TCT GAC GGG GTT CAG TCT CTT ATC GAG AAG GGT CAC   192
Leu Thr Val Phe Ser Asp Gly Val Gln Ser Leu Ile Glu Lys Gly His

CGG AAA ATC ACC CCT TTC TTC ATC CCC TAT GCC ATT ACA AAC ATG GGG   240
Arg Lys Ile Thr Pro Phe Phe Ile Pro Tyr Ala Ile Thr Asn Met Gly

TCT GCC CTG CTC GCT ATC GAA TTT GGT CTC ATG GGC CCA AAC TAT TCA   288
Ser Ala Leu Leu Ala Ile Glu Phe Gly Leu Met Gly Pro Asn Tyr Ser

ATT TCC ACT GCA TGT GCC ACT TCC AAC TAC TGC TTC CAT GCT GCC GCT   336
Ile Ser Thr Ala Cys Ala Thr Ser Asn Tyr Cys Phe His Ala Ala Ala

AAT CAT ATC CGC CGT GGT GAG GCT GAT CTT ATG ATT GCT GGA GGC ACT   384
Asn His Ile Arg Arg Gly Glu Ala Asp Leu Met Ile Ala Gly Gly Thr
```

FIGURE 1
1 OF 4

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GAG GCC GCA ATC ATT CCA ATT GGG TTG GGA GGC TTT GTG GCT TGC AGG   432
Glu Ala Ala Ile Ile Pro Ile Gly Leu Gly Gly Phe Val Ala Cys Arg

GCT TTG TCT CAA AGG AAC GAT GAC CCG CAG ACT GCC TCT AGG CCC TGG   480
Ala Leu Ser Gln Arg Asn Asp Asp Pro Gln Thr Ala Ser Arg Pro Trp

GAT AAA GAC CGT GAT GGT TTT GTG ATG GGT GAA GGT GCT GGA GTG TTG   528
Asp Lys Asp Arg Asp Gly Phe Val Met Gly Glu Gly Ala Gly Val Leu

GTG ATG GAG AGC TTG GAA CAT GCA ATG AGA CGA GGA GCA CCG ATT ATT   576
Val Met Glu Ser Leu Glu His Ala Met Arg Arg Gly Ala Pro Ile Ile

GCA GAG TAT TTG GGA GGT GCA ATC AAC TGT GAT GCT TAT CAC ATG ACT   624
Ala Glu Tyr Leu Gly Gly Ala Ile Asn Cys Asp Ala Tyr His Met Thr

GAT CCA AGG GCT GAT GGT CTT GGT GTC TCT TCT TGC ATT GAG AGT AGC   672
Asp Pro Arg Ala Asp Gly Leu Gly Val Ser Ser Cys Ile Glu Ser Ser

CTT GAA GAT GCT GGC GTC TCA CCT GAA GAG GTC AAT TAC ATA AAT GCT   720
Leu Glu Asp Ala Gly Val Ser Pro Glu Glu Val Asn Tyr Ile Asn Ala

CAT GCG ACT TCT ACT CTA GCT GGG GAT CTC GCC GAG ATA AAT GCC ATC   768
His Ala Thr Ser Thr Leu Ala Gly Asp Leu Ala Glu Ile Asn Ala Ile
```

FIGURE 1
2 OF 4

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AAG AAG GTT TTC AAG AAC ACA AAG GAT ATC AAA ATT AAT GCA ACT AAG      816
Lys Lys Val Phe Lys Asn Thr Lys Asp Ile Lys Ile Asn Ala Thr Lys

TCA ATG ATC GGA CAC TGT CTT GGA GCA TCT GGA GGT CTT GAA GCT ATA      864
Ser Met Ile Gly His Cys Leu Gly Ala Ser Gly Gly Leu Glu Ala Ile

GCG ACT ATT AAG GGA ATA AAC ACC GGC TGG CTT CAT CCC AGC ATT AAT      912
Ala Thr Ile Lys Gly Ile Asn Thr Gly Trp Leu His Pro Ser Ile Asn

CAA TTC AAT CCT GAG CCA TCG GTG GAG TTC GAC ACT GTT GCC AAC AAG      960
Gln Phe Asn Pro Glu Pro Ser Val Glu Phe Asp Thr Val Ala Asn Lys

AAG CAG CAA CAC GAA GTT AAC GTT GCG ATC TCG AAT TCA TTC GGA TTT     1008
Lys Gln Gln His Glu Val Asn Val Ala Ile Ser Asn Ser Phe Gly Phe

GGA GGC CAC AAC TCA GTC GTG GCT TTC TCG GCT TTC AAG CCA TGATTA      1056
Gly Gly His Asn Ser Val Val Ala Phe Ser Ala Phe Lys Pro

CCCATTTCAC AAGGTACTTG TCATTGAGAA TACGGATTAT GGACTTGCAG AGTAATTTCC  1116

CCATGTTTGT CGGAAGAGCA TATTACCACG GTTGTCCGTC AAACCCATTT AGGATACTGT  1176
```

FIGURE 1
3 OF 4

TCTATGTAAT AAAACTAAGG ATTATTAATT TCCCTTTTAA TCCTGTCTCC AGTTTGAGCA 1236

TGAAATTATA TTTATTTTAT CTTAGAAAGG TCAAATAAGA TTTTGTTTTA CCTCTGTAAA 1296

ACTTTTGTTT GTATTGGAAA GGAAGTGCCG TCTCAAAAAA AAAAAAAAAA AA 1348

FIGURE 1
4 OF 4

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Sequence Range: 1 to 1704

                         10           20          30          40
AAA TTA ACC CTC ACT AAA GGG AAC AAA AGC TGG AGC TCC ACC GNG GTG
Lys Leu Thr Leu Thr Lys Gly Asn Lys Ser Trp Ser Ser Thr Xxx Val>

50          60           70          80          90
             *
GCG GCC GCT CTA GAA CTA GTG GAT CCC CCG GGC TGC AGG AAT TCG GCA
Ala Ala Ala Leu Glu Leu Val Asp Pro Pro Gly Cys Arg Asn Ser Ala>

  100         110         120          130         140
                            *
CGA GCC GGC ATG GGC CTC GTC TCC GTA TTC GGC TCC GAC GTC GAC TCT
Arg Ala Gly Met Gly Leu Val Ser Val Phe Gly Ser Asp Val Asp Ser>

    150          160         170         180          190
                                           *
TAT TAC GAA AAG CTC CTC TCC GGC GAG AGC GGG ATC AGC TTA ATC GAC
Tyr Tyr Glu Lys Leu Leu Ser Gly Glu Ser Gly Ile Ser Leu Ile Asp>

       200         210          220         230         240
                                                          *
CGC TTC GAC GCT TCC AAG TTC CCC ACC AGG TTC GGC GGC CAG ATC CGG
Arg Phe Asp Ala Ser Lys Phe Pro Thr Arg Phe Gly Gly Gln Ile Arg>

          250         260         270          280
GGA TTC AAC GCG ACG GGA TAC ATC GAC GGG AAG AAC GAC AGG AGG CTC
Gly Phe Asn Ala Thr Gly Tyr Ile Asp Gly Lys Asn Asp Arg Arg Leu>

90          300          310         320         330
             *
GAC GAT TGC CTC CGC TAC TGC ATT GTC GCC GGG AAG AAG GCT CTC GAA
Asp Asp Cys Leu Arg Tyr Cys Ile Val Ala Gly Lys Lys Ala Leu Glu>
```

FIGURE 2
1/5

```
 340         350         360         370         380
                           *
AAT TCC GAT CTC GGC GGT GAA AGC CTC TCC AAG ATT GAT AAG GAG AGA
Asn Ser Asp Leu Gly Gly Glu Ser Leu Ser Lys Ile Asp Lys Glu Arg>

    390         400         410         420         430
                                          *
GCT GGA GTG CTA GTT GGA ACT GGT ATG GGT GGC CTA ACC GTC TTC TCT
Ala Gly Val Leu Val Gly Thr Gly Met Gly Gly Leu Thr Val Phe Ser>

        440         450         460         470         480
                                                          *
GAC GGG GTT CAG AAT CTC ATC GAG AAA GGT CAC CGG AAG ATC TCC CCG
Asp Gly Val Gln Asn Leu Ile Glu Lys Gly His Arg Lys Ile Ser Pro>

            490         500         510         520
TTT TTC ATT CCC TAT GCC ATT ACA AAC ATG GGG TCT GCT CTG CTT GCC
Phe Phe Ile Pro Tyr Ala Ile Thr Asn Met Gly Ser Ala Leu Leu Ala>

30              540         550         560         570
                  *
ATC GAT TTG GGT CTG ATG GGC CCA AAC TAT TCG ATT TCA ACT GCA TGT
Ile Asp Leu Gly Leu Met Gly Pro Asn Tyr Ser Ile Ser Thr Ala Cys>

 580         590         600         610         620
                           *
GCT ACT TCC AAC TAC TGC TTT TAT GCC GCT GCC AAT CAT ATC CGC CGA
Ala Thr Ser Asn Tyr Cys Phe Tyr Ala Ala Ala Asn His Ile Arg Arg>

    630         640         650         660         670
                                          *
GGC GAG GCT GAC CTC ATG ATT GCT GGA GGA ACT GAG GCT GCA ATC ATT
Gly Glu Ala Asp Leu Met Ile Ala Gly Gly Thr Glu Ala Ala Ile Ile>
```

FIGURE 2
2/5

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      680         690         700         710         720
                                                        *
CCA ATT GGG TTA GGA GGA TTC GTT GCC TGC AGG GCT TTA TCT CAA AGG
Pro Ile Gly Leu Gly Gly Phe Val Ala Cys Arg Ala Leu Ser Gln Arg>

        730         740         750         760
AAT GAT GAC CCT CAG ACT GCC TCA AGG CCG TGG GAT AAG GAC CGT GAT
Asn Asp Asp Pro Gln Thr Ala Ser Arg Pro Trp Asp Lys Asp Arg Asp>

70         780         790         800         810
             *
GGT TTT GTG ATG GGC GAA GGG GCT GGA GTA TTG GTT ATG GAG AGC TTG
Gly Phe Val Met Gly Glu Gly Ala Gly Val Leu Val Met Glu Ser Leu>

  820         830         840         850         860
                            *
GAA CAT GCA ATG AAA CGA GGA GCG CCG ATT ATT GCA GAA TAT TTG GGA
Glu His Ala Met Lys Arg Gly Ala Pro Ile Ile Ala Glu Tyr Leu Gly>

    870         880         890         900         910
                                          *
GGT GCA GTC AAT TGT GAT GCT TAT CAT ATG ACT GAT CCA AGG GCT GAT
Gly Ala Val Asn Cys Asp Ala Tyr His Met Thr Asp Pro Arg Ala Asp>

       920         930         940         950         960
                                                         *
GGG CTT GGT GTC TCC TCT TGC ATT GAG AGC AGT CTG GAA GAT GCT GGG
Gly Leu Gly Val Ser Ser Cys Ile Glu Ser Ser Leu Glu Asp Ala Gly>

          970         980         990        1000
GTC TCA CCT GAA GAG GTC AAT TAC ATA AAT GCT CAT GCG ACT TCC ACT
Val Ser Pro Glu Glu Val Asn Tyr Ile Asn Ala His Ala Thr Ser Thr>
```

FIGURE 2
3/5

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10          1020        1030        1040        1050
             *
CTT GCT GGG GAT CTT GCC GAG ATA AAT GCC ATC AAG AAG GTT TTC AAG
Leu Ala Gly Asp Leu Ala Glu Ile Asn Ala Ile Lys Lys Val Phe Lys>

 1060        1070        1080        1090        1100
                          *
AAC ACC AAG GAA ATC ACA ATC AAT GCA ACT AAG TCG ATG ATC GGA CAC
Asn Thr Lys Glu Ile Thr Ile Asn Ala Thr Lys Ser Met Ile Gly His>

  1110        1120        1130        1140        1150
                                       *
TGT CTT GGA GCA TCA GGG GGT CTT GAA GCC ATT GCG ACA ATT AAG GGA
Cys Leu Gly Ala Ser Gly Gly Leu Glu Ala Ile Ala Thr Ile Lys Gly>

     1160        1170        1180        1190        1200
                                                        *
ATA ACC ACC GGC TGG CTT CAT CCC AGC ATA AAC CAA TTC AAT CCC GAG
Ile Thr Thr Gly Trp Leu His Pro Ser Ile Asn Gln Phe Asn Pro Glu>

        1210        1220        1230        1240
CCA TCA GTG GAA TTC GAC ACA GTT GCC AAC AAG AAG CAG CAA CAT GAA
Pro Ser Val Glu Phe Asp Thr Val Ala Asn Lys Lys Gln Gln His Glu>

50          1260        1270        1280        1290
             *
GTG AAT GTT GCT ATC TCA AAT TCA TTC GGA TTC GGA GGC CAC AAC TCA
Val Asn Val Ala Ile Ser Asn Ser Phe Gly Phe Gly Gly His Asn Ser>

 1300        1310        1320        1330        1340
                          *
GTT GTA GCT TTC TCA GCC TTC AAG CCA TGA TTA CTC GGT TCA AAT GCA
Val Val Ala Phe Ser Ala Phe Lys Pro
```

FIGURE 2
4/5

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AATTTGTTGC TGAGACAGTG AGCTTCAACT TGCAGAGCAA TTTTTTACAT GCCTTGTCGT
CGGAAGAGCG TAATACCGGG ATAGTTCCTT GATAGTTCAT TTAGGATGTT TTACTGCAAT
AATCGAAGAT TATTTCCATT CTAATCCAGT CTCCGNCGAG TTTGAGAATC TATCTGTTTG
TATTAGAAAG AACGAGGCAA GATTTTGTTT CATGTTTGTG TTTGTATTAC TTTCTTTTTG
CCCTTGTCAA TGGCATTTAA GATAAGCTTA TAAAAAAAAA AAAAAAAAAA AAAACTCGAG
GGGGGGCCCG GTACCCAATT CGCCCTATAG TGAGTCGTAT GACAATTCAC TGTCCGTCGG
```

FIGURE 2
5/5

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        10         20         30         40         50         60
                                                                 *
ACTAAAGGGA ACAAAAGCTG GAGCTCCACC GCGGTGGCGG CCGCTCTAGA ACTAGTGGAT

        70         80         90        100        110        120
                                                                 *
CCCCCGGGCT GCAGGAATTC GGCACGAGTT TTCTTACTTG GGTCGGCTCA GCTCAGGTGT

          130             140            150             160
TCCA ATG GCG ACC GCT TCT TGC ATG GTT GCG TCC CCT TTC TGT ACG TGG
     Met Ala Thr Ala Ser Cys Met Val Ala Ser Pro Phe Cys Thr Trp

170             180            190              200            210
                 *
CTC GTA GCT GCA TGC ATG CCC ACT TCA TCC GAC AAC GAC CCA CGT TCC
Leu Val Ala Ala Cys Met Pro Thr Ser Ser Asp Asn Asp Pro Arg Ser

220             230            240            250             260
                                 *
CTT TCC CAC AAG CGG CTC CGC CTC TCC CGT CGC CGG AGG ACT CTC TCC
Leu Ser His Lys Arg Leu Arg Leu Ser Arg Arg Arg Arg Thr Leu Ser

   270            280             290            300            310
                                                  *
TCC CAT TGC TCC CTC CGC GGA TCC ACC TTC CAA TGC CTC GAT CCT TGC
Ser His Cys Ser Leu Arg Gly Ser Thr Phe Gln Cys Leu Asp Pro Cys

      320            330            340             350            360
                                                                    *
AAC CAG CAA CGC TTC CTC GGG GAT AAC GGA TTC GCT TCC CTC TTC GGA
Asn Gln Gln Arg Phe Leu Gly Asp Asn Gly Phe Ala Ser Leu Phe Gly
```

FIGURE 3 1/6

```
           370           380          390          400
TCC AAG CCT CTT CGT TCA AAT CGC GGC CAC CTG AGG CTC GGC CGC ACT
Ser Lys Pro Leu Arg Ser Asn Arg Gly His Leu Arg Leu Gly Arg Thr

410          420          430           440          450
              *
TCC CAT TCC GGG GAG GTC ATG GCT GTG GCT ATG CAA CCT GCA CAG GAA
Ser His Ser Gly Glu Val Met Ala Val Ala Met Gln Pro Ala Gln Glu

460           470          480          490           500
                            *
GTC TCC ACA AAT AAG AAA CCT GCT ACC AAG CAA AGG CGA GTA GTT GTG
Val Ser Thr Asn Lys Lys Pro Ala Thr Lys Gln Arg Arg Val Val Val

   510          520           530          540          550
                                            *
ACA GGT ATG GGC GTG GTG ACT CCT CTA GGC CAT GAC CCC GAT GTT TAC
Thr Gly Met Gly Val Val Thr Pro Leu Gly His Asp Pro Asp Val Tyr

      560          570          580           590          600
                                                            *
TAC AAC AAT CTC CTA GAC GGA ATA AGT GGC ATA AGT GAG ATA GAG AAC
Tyr Asn Asn Leu Leu Asp Gly Ile Ser Gly Ile Ser Glu Ile Glu Asn

        610           620          630          640
TTC GAC TGC TCT CAG TTT CCC ACG AGA ATT GCC GGA GAG ATC AAG TCT
Phe Asp Cys Ser Gln Phe Pro Thr Arg Ile Ala Gly Glu Ile Lys Ser

650          660          670           680          690
              *
TTT TCC ACA GAT GGC TGG GTG GCC CCA AAG TTC TCC GAG AGG ATG GAC
Phe Ser Thr Asp Gly Trp Val Ala Pro Lys Phe Ser Glu Arg Met Asp
```

FIGURE 3 2 OF 6

```
    700         710         720         730         740
                  *
AAG TTC ATG CTT TAC ATG CTG ACT GCA GGC AAG AAA GCA TTA GCA GAT
Lys Phe Met Leu Tyr Met Leu Thr Ala Gly Lys Lys Ala Leu Ala Asp

    750         760         770         780         790
                                          *
GGT GGA ATC ACT GAA GAT GCG ATG AAA GAG CTC AAT AAA AGA AAG TGT
Gly Gly Ile Thr Glu Asp Ala Met Lys Glu Leu Asn Lys Arg Lys Cys

        800         810         820         830         840
                                                          *
GGA GTT CTC ATT GGC TCC GGA TTG GGC GGT ATG AAG GTA TTC AGC GAT
Gly Val Leu Ile Gly Ser Gly Leu Gly Gly Met Lys Val Phe Ser Asp

            850         860         870         880
TCC ATT GAA GCT CTG AGG ACT TCA TAT AAG AAG ATC AGT CCC TTT TGT
Ser Ile Glu Ala Leu Arg Thr Ser Tyr Lys Lys Ile Ser Pro Phe Cys

890         900         910         920         930
              *
GTA CCT TTT TCT ACC ACA AAT ATG GGA TCC GCT ATT CTT GCA ATG GAC
Val Pro Phe Ser Thr Thr Asn Met Gly Ser Ala Ile Leu Ala Met Asp

940         950         960         970         980
                          *
TTG GGA TGG ATG GGC CCT AAC TAT TCG ATA TCA ACT GCC TGT GCA ACA
Leu Gly Trp Met Gly Pro Asn Tyr Ser Ile Ser Thr Ala Cys Ala Thr

    990         1000        1010        1020        1030
                                          *
AGT AAC TTC TGT ATA CTG AAT GCT GCG AAC CAC ATA ATC AAA GGC GAA
Ser Asn Phe Cys Ile Leu Asn Ala Ala Asn His Ile Ile Lys Gly Glu
```

FIGURE 3 3 OF 6

```
      1040        1050        1060        1070        1080
          *
GCA GAC ATG ATG CTT TGT GGT GGC TCG GAT GCG GCC GTT TTA CCT GTT
Ala Asp Met Met Leu Cys Gly Gly Ser Asp Ala Ala Val Leu Pro Val

       1090        1100        1110        1120
GGT TTG GGA GGT TTC GTA GCA TGC CGA GCT TTG TCA CAG AGG AAT AAT
Gly Leu Gly Gly Phe Val Ala Cys Arg Ala Leu Ser Gln Arg Asn Asn

1130        1140        1150        1160        1170
              *
GAC CCT ACC AAA GCT TCG AGA CCA TGG GAC AGT AAT CGT GAT GGA TTT
Asp Pro Thr Lys Ala Ser Arg Pro Trp Asp Ser Asn Arg Asp Gly Phe

  1180        1190        1200        1210        1220
                             *
GTG ATG GGA GAA GGA GCT GGA GTT TTA CTT CTT GAG GAG TTA GAG CAT
Val Met Gly Glu Gly Ala Gly Val Leu Leu Leu Glu Glu Leu Glu His

    1230        1240        1250        1260        1270
                                           *
GCA AAG AAA AGA GGT GCA ACC ATT TAT GCG GAA TTT CTA GGT GGG AGT
Ala Lys Lys Arg Gly Ala Thr Ile Tyr Ala Glu Phe Leu Gly Gly Ser

       1280        1290        1300        1310        1320
                                                          *
TTC ACT TGC GAC GCC TAC CAC ATG ACC GAG CCT CAC CCT GAA GGA GCT
Phe Thr Cys Asp Ala Tyr His Met Thr Glu Pro His Pro Glu Gly Ala

         1330        1340        1350        1360
GGT GTG ATC CTC TGC ATA GAG AAG GCC TTG GCT CAG TCC GGA GTC TCG
Gly Val Ile Leu Cys Ile Glu Lys Ala Leu Ala Gln Ser Gly Val Ser
```

FIGURE 3 4 OF 6

```
1370        1380        1390        1400        1410
              *
AGG GAA GAC GTA AAT TAC ATA AAT GCG CAT GCA ACT TCC ACT CCT GCT
Arg Glu Asp Val Asn Tyr Ile Asn Ala His Ala Thr Ser Thr Pro Ala

 1420        1430        1440        1450        1460
                          *
GGA GAT ATC AAG GAA TAC CAA GCT CTC GCC CAC TGT TTC GGC CAA AAC
Gly Asp Ile Lys Glu Tyr Gln Ala Leu Ala His Cys Phe Gly Gln Asn

   1470        1480        1490        1500        1510
                                      *
AGT GAG CTG AGA GTG AAT TCC ACC AAA TCG ATG ATC GGT CAC CTT CTT
Ser Glu Leu Arg Val Asn Ser Thr Lys Ser Met Ile Gly His Leu Leu

     1520        1530        1540        1550        1560
                                                      *
GGA GGA GCT GGT GGC GTA GAA GCA GTT GCA GTA GTT CAG GCA ATA AGG
Gly Gly Ala Gly Gly Val Glu Ala Val Ala Val Val Gln Ala Ile Arg

      1570        1580        1590        1600
ACA GGA TGG ATC CAT CCA AAT ATT AAT TTG GAA GAC CCG GAC GAA GGC
Thr Gly Trp Ile His Pro Asn Ile Asn Leu Glu Asp Pro Asp Glu Gly

1610        1620        1630        1640        1650
              *
GTG GAT GCA AAA CTG CTC GTC GGC CCT AAG AAG GAG AAA CTG AAG GTC
Val Asp Ala Lys Leu Leu Val Gly Pro Lys Lys Glu Lys Leu Lys Val

 1660        1670        1680        1690        1700
                          *
AAG GTC GGT TTG TCC AAT TCA TTT GGG TTC GGC GGC CAT AAC TCA TCC
Lys Val Gly Leu Ser Asn Ser Phe Gly Phe Gly Gly His Asn Ser Ser
```

FIGURE 3 5 OF 6

```
1710          1720            1730         1740         1750         1760
                                              *
ATA CTA TTT GCC CCC TGC AAC TAG A AAAGAGTCTG TGGAAGCCGA GAGTCTTTGA
Ile Leu Phe Ala Pro Cys Asn ***

     1770         1780         1790         1800         1810         1820
                                              *
GAACTCATGC ACGTTAGTAG CTTCTTATGC CTCTGAAACC GAGATAGACC GGCTACTCGA

     1830         1840         1850         1860         1870         1880
                                              *
GGGGATGCCA AAGATACTCC TTGCCGGTAT TGGTGTTAAG AGATCACTGC TTGTCCCTTT

     1890         1900         1910         1920         1930         1940
                                              *
TATTTTCTTC TTCTTTTGAG AGCTTTAACC GAGGTAGTCG TATTTTCGAG CTTTTCGAAT

     1950         1960         1970         1980         1990         2000
                                              *
ACATGTTCGT TATCGGATCA ATGTGTTTCT TCTAAGATCA TTTGTAATGC ATATTTTGAA

     2010         2020         2030         2040
                                              *
AAACCACATC TCAGTATGCA AAATAAAAAA AAAAAAAAAA AAAAAA
```

FIGURE 3 6 OF 6

```
Sequence Range: 1 to 1921

        10         20         30         40         50         60
                                                                  *
CGGCACGAGG TCACCTCTTA CCTCGCCTGC TTCGAGCCCT GCCATGACTA CTACACCTCC

        70         80         90        100        110        120
                                                                  *
GCATCCTTGT TCGGATCCAG GCCCATCCGC ACCACCCGCA GGCACCGGAG GCTCAATCGA

       130        140        150        160        170        180
                                                                  *
GCTTCCCCTT CCGGGGAGGC AATGGCTGTG GCTCTGCAAC CTGCACAGGA AGTTACCACA

           190         200         210         220
AAG AAG AAG CCA AGT ATC AAA CAG CGG CGA GTA GTT GTG ACT GGA ATG
Lys Lys Lys Pro Ser Ile Lys Gln Arg Arg Val Val Val Thr Gly Met>

230         240         250         260         270
              *
GGT GTG GTG ACT CCT CTA GGC CAT GAC CCT GAT GTT TTC TAC AAT AAT
Gly Val Val Thr Pro Leu Gly His Asp Pro Asp Val Phe Tyr Asn Asn>

   280         290         300         310         320
                             *
CTG CTT GAT GGA ACG AGT GGC ATA AGT GAG ATA GAG ACC TTT GAT TGT
Leu Leu Asp Gly Thr Ser Gly Ile Ser Glu Ile Glu Thr Phe Asp Cys>

     330         340         350         360         370
                                           *
GCT CAA TTT CCT ACG AGA ATT GCT GGA GAG ATC AAG TCT TTC TCC ACA
Ala Gln Phe Pro Thr Arg Ile Ala Gly Glu Ile Lys Ser Phe Ser Thr>
```

FIGURE 4
1/6

```
        380         390         400         410         420
                                                          *
GAT GGT TGG GTG GCC CCG AAG CTC TCC AAG AGG ATG GAC AAG TTC ATG
Asp Gly Trp Val Ala Pro Lys Leu Ser Lys Arg Met Asp Lys Phe Met>

          430         440         450         460
CTT TAC ATG CTG ACT GCC GGC AAG AAA GCA TTA ACA AAT GGT GGA ATC
Leu Tyr Met Leu Thr Ala Gly Lys Lys Ala Leu Thr Asn Gly Gly Ile>

470         480         490         500         510
              *
ACC GAA GAT GTG ATG AAA GAG CTA GAT AAA AGA AAA TGC GGA GTT CTC
Thr Glu Asp Val Met Lys Glu Leu Asp Lys Arg Lys Cys Gly Val Leu>

  520         530         540         550         560
                            *
ATT GGC TCA GCA ATG GGT GGA ATG AAG GTA TTC AAT GAT GCC ATT GAA
Ile Gly Ser Ala Met Gly Gly Met Lys Val Phe Asn Asp Ala Ile Glu>

    570         580         590         600         610
                                          *
GCC CTA AGG ATT TCA TAT AAG AAG ATG AAT CCC TTT TGT GTA CCT TTC
Ala Leu Arg Ile Ser Tyr Lys Lys Met Asn Pro Phe Cys Val Pro Phe>

       620         630         640         650         660
                                                         *
GCT ACC ACA AAT ATG GGA TCA GCT ATG CTT GCA ATG GAC TTG GGA TGG
Ala Thr Thr Asn Met Gly Ser Ala Met Leu Ala Met Asp Leu Gly Trp>

          670         680         690         700
ATG GGC CCC AAC TAC TCG ATA TCT ACT GCT TGT GCA ACG AGT AAC TTT
Met Gly Pro Asn Tyr Ser Ile Ser Thr Ala Cys Ala Thr Ser Asn Phe>
```

FIGURE 4
2/6

```
710           720           730           740           750
               *
TGT ATC CTG AAT GCT GCG AAC CAC ATA ATC AGA GGC GAA GCA GAT GTG
Cys Ile Leu Asn Ala Ala Asn His Ile Ile Arg Gly Glu Ala Asp Val>

   760           770           780           790           800
                                 *
ATG CTT TGC GGG GGC TCA GAT GCG GTA ATC ATA CCT ATT GGT ATG GGA
Met Leu Cys Gly Gly Ser Asp Ala Val Ile Ile Pro Ile Gly Met Gly>

      810           820           830           840           850
                                                  *
GGT TTT GTT GCA TGC CGA GCT TTG TCA CAG AGA AAT GCC GAC CCT ACT
Gly Phe Val Ala Cys Arg Ala Leu Ser Gln Arg Asn Ala Asp Pro Thr>

         860           870           880           890           900
                                                                   *
AAA GCT TCA AGA CCA TGG GAC AGT AAT CGT GAT GGA TTT GTT ATG GGG
Lys Ala Ser Arg Pro Trp Asp Ser Asn Arg Asp Gly Phe Val Met Gly>

            910           920           930           940
GAA GGA GCT GGA GTG CTA CTA CTA GAG GAG TTA GAG CAT GCA AAG AAA
Glu Gly Ala Gly Val Leu Leu Leu Glu Glu Leu Glu His Ala Lys Lys>

950           960           970           980           990
               *
AGA GGT GCG ACT ATT TAC GCA GAA TTT CTA GGT GGA AGT TTC ACT TGC
Arg Gly Ala Thr Ile Tyr Ala Glu Phe Leu Gly Gly Ser Phe Thr Cys>

  1000          1010          1020          1030          1040
                                *
GAT GCC TAC CAC ATG ACC GAG CCT CAC CCT GAT GGA GCT GGA GTG ATT
Asp Ala Tyr His Met Thr Glu Pro His Pro Asp Gly Ala Gly Val Ile>
```

FIGURE 4
3/6

```
   1050         1060        1070        1080        1090
                                           *
CTC TGC ATA GAG AAG GCT TTG GCT CAG TCA GGA GTC TCT AGG GAA GAC
Leu Cys Ile Glu Lys Ala Leu Ala Gln Ser Gly Val Ser Arg Glu Asp>

      1100        1110        1120        1130        1140
                                                         *
GTA AAT TAC ATA AAT GCA CAT GCC ACA TCC ACT CCA GCT GGA GAT ATC
Val Asn Tyr Ile Asn Ala His Ala Thr Ser Thr Pro Ala Gly Asp Ile>

         1150        1160        1170         1180
AAA GAG TAC CAA GCT CTT ATC CAC TGT TTC GGC CAA AAC AAC GAG TTA
Lys Glu Tyr Gln Ala Leu Ile His Cys Phe Gly Gln Asn Asn Glu Leu>

1190        1200        1210        1220        1230
              *
AAA GTG AAT TCT ACC AAA TCA ATG ATT GGT CAC CTT CTC GGA GCA GCC
Lys Val Asn Ser Thr Lys Ser Met Ile Gly His Leu Leu Gly Ala Ala>

  1240        1250        1260        1270        1280
                             *
GGT GGT GTG GAA GCA GTT TCA GTA GTT CAG GCA ATA AGG ACT GGG TGG
Gly Gly Val Glu Ala Val Ser Val Val Gln Ala Ile Arg Thr Gly Trp>

   1290         1300        1310        1320        1330
                                           *
ATC CAT CCG AAT ATT AAT TTG GAA AAC CCA GAT GAA GGC GTG GAT ACC
Ile His Pro Asn Ile Asn Leu Glu Asn Pro Asp Glu Gly Val Asp Thr>

      1340        1350        1360        1370        1380
                                                         *
AAA TTG CTC GTG GGC CCT AAG AAG GAG AGA CTG AAC ATT AAG GTC GGT
Lys Leu Leu Val Gly Pro Lys Lys Glu Arg Leu Asn Ile Lys Val Gly>
```

FIGURE 4
4/6

```
        1390        1400        1410        1420
TTG TCT AAT TCA TTC GGG TTT GGT GGG CAC AAC TCG TCC ATA CTC TTC
Leu Ser Asn Ser Phe Gly Phe Gly Gly His Asn Ser Ser Ile Leu Phe>

1430        1440       1450       1460       1470       1480
              *
GCC CCT TAC AAC TAG GGCGTTT CATGTGTGGA ATTCTACTCA ATCTATCAAA
Ala Pro Tyr Asn ***>

      1490       1500       1510       1520       1530       1540
                    *
GCTGAAGTTT TGAGGACTCC AGCATGTTGG TAGCTCCTTA CGTCTCTAGA CATGCCCATG

      1550       1560       1570       1580       1590       1600
                    *
AGTTTTGTGT CGGGAGCTGT AGTCGGAACC ATGACGGATT GAGTACTCAT GGCGACACAG

      1610       1620       1630       1640       1650       1660
                    *
GATATACTCC TTGCTAGAAT TGTTAGAGCA CTATTCATTA TCCCATTTTT TTTCTGAAAT

      1670       1680       1690       1700       1710       1720
                    *
CTCCCTCCTT ACGGTAGTTG TACTTTCGAG CGTTTCATCG AGTCAGTGAA GAAGAGAACA

      1730       1740       1750       1760       1770       1780
                    *
AAGCTAACTC GGGCACGTAG TAACCATTTG CCCTTTGTTT TGCTCTCTAT TTTATCGCCG

      1790       1800       1810       1820       1830       1840
                    *
TTTTGTGGGT TAAAATTTGT AAAACTAGAC GACTGGTTTG TTTTCTCTTG ATCATTGGAG
```

FIGURE 4
5/6

```
      1850       1860       1870       1880       1890       1900
                    *
ATGTATGGCC ATATTTGCCT TTCATTGATG ATAAAAAAAA AAAAAAAAAA AAAAAAAAAA

      1910       1920
                    *
AAAAAAAAAA AAAAAAAAAA A
```

FIGURE 4
6/6

```
CTGGTACGCC TGCAGGTACC GGTCCGGAAT TCCCGGGTCG ACCCACGCGT CCGTCTTCCC   60

ACTCCGATCG TTCTTCTTCC ACCGCATCTC TTCTCTTCTC TTGGCTTCTC CGCCATCCTC  120

CGCCGCC ATG CAT TCC CTC CAG TCA CCC TCC CTT CGG GCC TCC CCG CTC    169
        Met His Ser Leu Gln Ser Pro Ser Leu Arg Ala Ser Pro Leu
         1               5                  10

GAC CCC TTC CGC CCC AAA TCA TCC ACC GTC CGC CCC CTC CAC CGA GCA    217
Asp Pro Phe Arg Pro Lys Ser Ser Thr Val Arg Pro Leu His Arg Ala
15                  20                  25                  30

TCA ATT CCC AAC GTC CGG GCC GCT TCC CCC ACC GTC TCC GCT CCC AAG    265
Ser Ile Pro Asn Val Arg Ala Ala Ser Pro Thr Val Ser Ala Pro Lys
                35                  40                  45

CGC GAG ACC GAC CCC AAG AAG CGC GTC GTG ATC ACC GGA ATG GGC CTT    313
Arg Glu Thr Asp Pro Lys Lys Arg Val Val Ile Thr Gly Met Gly Leu
            50                  55                  60

GTC TCC GTT TTC GGC TCC GAC GTC GAT GCG TAC TAC GAC AAG CTC CTG    361
Val Ser Val Phe Gly Ser Asp Val Asp Ala Tyr Tyr Asp Lys Leu Leu
        65                  70                  75

TCA GGC GAG AGC GGG ATC GGC CCA ATC GAC CGC TTC GAC GCC TCC AAG    409
Ser Gly Glu Ser Gly Ile Gly Pro Ile Asp Arg Phe Asp Ala Ser Lys
    80                  85                  90

TTC CCC ACC AGG TTC GGC GGC CAG ATT CGT GGC TTC AAC TCC ATG GGA    457
Phe Pro Thr Arg Phe Gly Gly Gln Ile Arg Gly Phe Asn Ser Met Gly
95                  100                 105                 110

TAC ATT GAC GGC AAA AAC GAC AGG CGG CTT GAT GAT TGC CTT CGC TAC    505
Tyr Ile Asp Gly Lys Asn Asp Arg Arg Leu Asp Asp Cys Leu Arg Tyr
                115                 120                 125
```

FIGURE 5
1/4

```
TGC ATT GTC GCC GGG AAG AAG TCT CTT GAG GAC GCC GAT CTC GGT GCC    553
Cys Ile Val Ala Gly Lys Lys Ser Leu Glu Asp Ala Asp Leu Gly Ala
            130                 135                 140

GAC CGC CTC TCC AAG ATC GAC AAG GAG AGA GCC GGA GTG CTG GTT GGG    601
Asp Arg Leu Ser Lys Ile Asp Lys Glu Arg Ala Gly Val Leu Val Gly
        145                 150                 155

ACA GGA ATG GGT GGT CTG ACT GTC TTC TCT GAC GGG GTT CAA TCT CTT    649
Thr Gly Met Gly Gly Leu Thr Val Phe Ser Asp Gly Val Gln Ser Leu
    160                 165                 170

ATC GAG AAG GGT CAC CGG AAA ATC ACC CCT TTC TTC ATC CCC TAT GCC    697
Ile Glu Lys Gly His Arg Lys Ile Thr Pro Phe Phe Ile Pro Tyr Ala
175                 180                 185                 190

ATT ACA AAC ATG GGG TCT GCC CTG CTC GCT ATT GAA CTC GGT CTG ATG    745
Ile Thr Asn Met Gly Ser Ala Leu Leu Ala Ile Glu Leu Gly Leu Met
                195                 200                 205

GGC CCA AAC TAT TCA ATT TCC ACT GCA TGT GCC ACT TCC AAC TAC TGC    793
Gly Pro Asn Tyr Ser Ile Ser Thr Ala Cys Ala Thr Ser Asn Tyr Cys
            210                 215                 220

TTC CAT GCT GCT GCT AAT CAT ATC CGC CGT GGT GAG GCT GAT CTT ATG    841
Phe His Ala Ala Ala Asn His Ile Arg Arg Gly Glu Ala Asp Leu Met
        225                 230                 235

ATT GCT GGA GGC ACT GAG GCC GCA ATC ATT CCA ATT GGG TTG GGA GGC    889
Ile Ala Gly Gly Thr Glu Ala Ala Ile Ile Pro Ile Gly Leu Gly Gly
    240                 245                 250
```

FIGURE 5
2/4

```
TTT GTG GCT TGC AGG GCT CTG TCT CAA AGG AAC GAT GAC CCT CAG ACT     937
Phe Val Ala Cys Arg Ala Leu Ser Gln Arg Asn Asp Asp Pro Gln Thr
255                 260                 265                 270

GCC TCT AGG CCC TGG GAT AAA GAC CGT GAT GGT TTT GTG ATG GGT GAA     985
Ala Ser Arg Pro Trp Asp Lys Asp Arg Asp Gly Phe Val Met Gly Glu
                275                 280                 285

GGT GCT GGA GTG TTG GTG CTG GAG AGC TTG GAA CAT GCA ATG AAA CGA    1033
Gly Ala Gly Val Leu Val Leu Glu Ser Leu Glu His Ala Met Lys Arg
            290                 295                 300

GGA GCA CCT ATT ATT GCA GAG TAT TTG GGA GGT GCA ATC AAC TGT GAT    1081
Gly Ala Pro Ile Ile Ala Glu Tyr Leu Gly Gly Ala Ile Asn Cys Asp
        305                 310                 315

GCT TAT CAC ATG ACT GAC CCA AGG GCT GAT GGT CTC GGT GTC TCC TCT    1129
Ala Tyr His Met Thr Asp Pro Arg Ala Asp Gly Leu Gly Val Ser Ser
    320                 325                 330

TGC ATT GAG AGT AGC CTT GAA GAT GCT GGC GTC TCA CCT GAA GAG GTC    1176
Cys Ile Glu Ser Ser Leu Glu Asp Ala Gly Val Ser Pro Glu Glu Val
335                 340                 345                 350

AAT TAC ATA AAT GCT CAT GCG ACT TCT ACT CTA GCT GGG GAT CTC GCC    1224
Asn Tyr Ile Asn Ala His Ala Thr Ser Thr Leu Ala Gly Asp Leu Ala
                355                 360                 365

GAG ATA AAT GCC ATC AAG AAG GTT TTC AAG AAC ACA AAG GAT ATC AAA    1272
Glu Ile Asn Ala Ile Lys Lys Val Phe Lys Asn Thr Lys Asp Ile Lys
            370                 375                 380
```

FIGURE 5
3/4

```
ATT AAT GCA ACT AAG TCA ATG ATC GGA CAC TGT CTT GGA GCC TCT GGA    1320
Ile Asn Ala Thr Lys Ser Met Ile Gly His Cys Leu Gly Ala Ser Gly
        385                 390                 395

GGT CTT GAA GCT ATA GCG ACT ATT AAG GGA ATA AAC ACC GGC TGG CTT    1368
Gly Leu Glu Ala Ile Ala Thr Ile Lys Gly Ile Asn Thr Gly Trp Leu
    400                 405                 410

CAT CCC AGC ATT AAT CAA TTC AAT CCT GAG CCA TCC GTG GAG TTC GAC    1416
His Pro Ser Ile Asn Gln Phe Asn Pro Glu Pro Ser Val Glu Phe Asp
415                 420                 425                 430

ACT GTT GCC AAC AAG AAG CAG CAA CAC GAA GTT AAT GTT GCG ATC TCG    1464
Thr Val Ala Asn Lys Lys Gln Gln His Glu Val Asn Val Ala Ile Ser
                435                 440                 445

AAT TCA TTT GGA TTC GGA GGC CAC AAC TCA GTC GTG GCT TTC TCG GCT    1512
Asn Ser Phe Gly Phe Gly Gly His Asn Ser Val Val Ala Phe Ser Ala
            450                 455                 460

TTC AAG CCA TGA TTACC CATTTCACAA GGCACTTGTC ATTGAGAGTA CGGTTGTTCG  1569
Phe Lys Pro
        465

TCAAACCCAT TTAGGATACT GTTCTATGTA AAAAAAGTA AGGATTATCA CTTTCCCTTC  1629

TAATCCTGTC TCCAGTTTGA GAATGAAATT ATATTTATTT TAAAAAAAAA AAAAAAGGGC  1689

GGCCGCTCTA GAGGATCCAA GCT                                          1712
```

FIGURE 5
4/4

```
Sequence Range: 1 to 1802

         10         20         30         40         50         60
                                                                   *
GGTCGACCCA CGCGTCCGGG CTTTCCGACC ACATTTCATT TCTTGCCTCG TTATCTCCGC

         70         80         90          100         110
CGCTCCTCCG CCGTCGTTCG CCGCCGCCGC C ATG CAA TCC CTC CAC TCC CCT TCC
                                   Met Gln Ser Leu His Ser Pro Ser

   120         130          140         150         160
     *
CTC CGC CCC TCC CCT CTC GAG CCC TTC CGC CTC AAT TCC CCC TCC TCC
Leu Arg Pro Ser Pro Leu Glu Pro Phe Arg Leu Asn Ser Pro Ser Ser

     170         180         190          200         210
                   *
GCC GCC GCT CTC CGC CCC CTC CGT CGC GCC AGC CTC CCC GTC ATC CGT
Ala Ala Ala Leu Arg Pro Leu Arg Arg Ala Ser Leu Pro Val Ile Arg

        220         230         240         250
                                  *
GCT GCC ACC GCC TCC GCC CCC AAG CGC GAG TCC GAC CCC AAG AAG CGG
Ala Ala Thr Ala Ser Ala Pro Lys Arg Glu Ser Asp Pro Lys Lys Arg

260         270          280         290         300
                                                   *
GTC GTC ATC ACC GGC ATG GGC CTC GTC TCC GTC TTC GGC TCC GAC GTC
Val Val Ile Thr Gly Met Gly Leu Val Ser Val Phe Gly Ser Asp Val

310         320         330         340          350
GAC GCC TAC TAC GAC AAG CTG CTC TCC GGC GAG AGC GGC ATC AGC CTA
Asp Ala Tyr Tyr Asp Lys Leu Leu Ser Gly Glu Ser Gly Ile Ser Leu
```

FIGURE 6
1/5

```
 360           370           380           390           400
  *
ATC GAC CGC TTC GAC GCT TCC AAA TTC CCC ACC AGG TTC GCC GGC CAG
Ile Asp Arg Phe Asp Ala Ser Lys Phe Pro Thr Arg Phe Ala Gly Gln

       410           420           430           440           450
                      *
ATC CGT GGC TTC AAC GCG ACG GGC TAC ATC GAC GGC AAG AAC GAC CGG
Ile Arg Gly Phe Asn Ala Thr Gly Tyr Ile Asp Gly Lys Asn Asp Arg

         460           470           480           490
                                      *
CGG CTC GAC GAT TGC CTC CGC TAC TGC ATT GTC GCC GGC AAG AAG GCT
Arg Leu Asp Asp Cys Leu Arg Tyr Cys Ile Val Ala Gly Lys Lys Ala

500           510           520           530           540
                                                         *
CTC GAA GAC GCC GAT CTC GCC GGC CAA TCC CTC TCC AAG ATT GAT AAG
Leu Glu Asp Ala Asp Leu Ala Gly Gln Ser Leu Ser Lys Ile Asp Lys

550           560           570           580           590
GAG AGG GCC GGA GTG CTA GTT GGA ACC GGT ATG GGT GGC CTA ACT GTC
Glu Arg Ala Gly Val Leu Val Gly Thr Gly Met Gly Gly Leu Thr Val

    600           610           620           630           640
     *
TTC TCT GAC GGG GTT CAG AAT CTC ATC GAG AAA GGT CAC CGG AAG ATC
Phe Ser Asp Gly Val Gln Asn Leu Ile Glu Lys Gly His Arg Lys Ile

        650           660           670           680           690
                       *
TCC CCG TTT TTC ATT CCA TAT GCC ATT ACA AAC ATG GGG TCT GCG CTG
Ser Pro Phe Phe Ile Pro Tyr Ala Ile Thr Asn Met Gly Ser Ala Leu
```

FIGURE 6
2/5

```
          700         710         720         730
                                    *
CTT GCC ATC GAT TTG GGT CTG ATG GGC CCA AAC TAT TCG ATT TCA ACT
Leu Ala Ile Asp Leu Gly Leu Met Gly Pro Asn Tyr Ser Ile Ser Thr

740         750         760         770         780
                                                  *
GCA TGT GCT ACT TCC AAC TAC TGC TTT TAT GCT GCC GCC AAT CAT ATC
Ala Cys Ala Thr Ser Asn Tyr Cys Phe Tyr Ala Ala Ala Asn His Ile

790         800         810         820         830
CGC CGA GGT GAG GCT GAC CTG ATG ATT GCT GGA GGA ACT GAG GCT GCG
Arg Arg Gly Glu Ala Asp Leu Met Ile Ala Gly Gly Thr Glu Ala Ala

    840         850         860         870         880
     *
GTC ATT CCA ATT GGT TTA GGA GGA TTC GTT GCC TGC AGG GCT TTA TCT
Val Ile Pro Ile Gly Leu Gly Gly Phe Val Ala Cys Arg Ala Leu Ser

        890         900         910         920         930
                     *
CAA AGG AAT GAT GAT CCT CAG ACT GCC TCA AGG CCG TGG GAT AAG GAC
Gln Arg Asn Asp Asp Pro Gln Thr Ala Ser Arg Pro Trp Asp Lys Asp

          940         950         960         970
                                    *
CGT GAT GGC TTT GTG ATG GGT GAA GGG GCT GGA GTA TTG GTT ATG GAG
Arg Asp Gly Phe Val Met Gly Glu Gly Ala Gly Val Leu Val Met Glu

980         990         1000        1010        1020
                                                  *
AGC TTG GAG CAT GCA ATG AAA CGG GGA GCG CCG ATT ATT GCA GAA TAT
Ser Leu Glu His Ala Met Lys Arg Gly Ala Pro Ile Ile Ala Glu Tyr
```

FIGURE 6
3/5

```
1030        1040        1050        1060        1070
TTG GGA GGT GCA GTC AAC TGT GAT GCT TAT CAT ATG ACT GAT CCA AGG
Leu Gly Gly Ala Val Asn Cys Asp Ala Tyr His Met Thr Asp Pro Arg

 1080        1090        1100        1110        1120
    *
GCT GAT GGG CTT GGT GTC TCC TCG TGC ATT GAG AGC AGT CTC GAA GAT
Ala Asp Gly Leu Gly Val Ser Ser Cys Ile Glu Ser Ser Leu Glu Asp

   1130        1140        1150        1160        1170
                  *
GCC GGG GTC TCA CCT GAA GAG GTC AAT TAC ATA AAT GCT CAT GCG ACT
Ala Gly Val Ser Pro Glu Glu Val Asn Tyr Ile Asn Ala His Ala Thr

     1180        1190        1200        1210
                                *
TCT ACT CTT GCT GGG GAT CTT GCC GAG ATA AAT GCC ATT AAG AAA GTT
Ser Thr Leu Ala Gly Asp Leu Ala Glu Ile Asn Ala Ile Lys Lys Val

1220        1230        1240        1250        1260
                                                    *
TTC AAG AAC ACC AAG GAA ATC AAA ATC AAT GCA ACT AAG TCA ATG ATC
Phe Lys Asn Thr Lys Glu Ile Lys Ile Asn Ala Thr Lys Ser Met Ile

1270        1280        1290        1300        1310
GGA CAC TGT CTT GGA GCA TCA GGA GGT CTT GAA GCC ATC GCA ACC ATT
Gly His Cys Leu Gly Ala Ser Gly Gly Leu Glu Ala Ile Ala Thr Ile

  1320        1330        1340        1350        1360
     *
AAG GGA ATA ACC ACC GGC TGG CTT CAT CCC AGC ATT AAT CAA TTT AAT
Lys Gly Ile Thr Thr Gly Trp Leu His Pro Ser Ile Asn Gln Phe Asn
```

FIGURE 6
4/5

```
    1370        1380        1390        1400        1410
                  *
CCC GAG CCA TCG GTG GAC TTC AAC ACT GTT GCC AAC AAA AAG CAG CAA
Pro Glu Pro Ser Val Asp Phe Asn Thr Val Ala Asn Lys Lys Gln Gln

     1420        1430        1440        1450
                               *
CAT GAA GTG AAC GTC GCT ATC TCG AAT TCT TTT GGA TTT GGA GGG CAC
His Glu Val Asn Val Ala Ile Ser Asn Ser Phe Gly Phe Gly Gly His

1460        1470        1480        1490        1500        1510
                                                   *
AAC TCG GTT GTG GCA TTC TCA GCT TTC AAG CCA TGA ATTCT ACTTGGTTCA
Asn Ser Val Val Ala Phe Ser Ala Phe Lys Pro ***

      1520       1530       1540       1550       1560       1570
                                                     *
AAATGCACAC CAGTTGCTGA GATAGGGCTT CAACTTGCAG AGCAATTTTT TAAATGCCTT

      1580       1590       1600       1610       1620       1630
                                                     *
GTCGGAAGAG CGTAATACCG GAATAGGTCG GTCCTTTGAT AGTTCCTCGA AGCCATTTAG

      1640       1650       1660       1670       1680       1690
                                                     *
GATGATGTTT TACTGTAATA ATCGAAGATG ATTCCCATTT TAAATCTAGT CTCTGATTTA

      1700       1710       1720       1730       1740       1750
                                                     *
TGTATTAGAA AGACCAATGA AAGATTTTGT GTCATGTTTG TGTTGTCAAT GTTATTTAAG

      1760       1770       1780       1790       1800
                                                     *
ATAAAGCAAA AAAAAAAAAA AAGGGCGGCC GCTCTAGAGG ATCCAGCTTA CT
```

FIGURE 6
5/5

```
Sequence Range: 1 to 2369

        10         20         30         40         50         60
                                                                   *
GTACGCCTGC AGGTACCGGT CCGGAATTCC CGGGTCGACC CACGCGTCCG CATAAAAGAG

        70         80         90        100        110        120
                                                                   *
AGAGAGAGGG ATCCATCGAA TGCGGCCACC CTCCTTTCAT CTTCGATTCA TTACCATACC

       130        140        150        160        170        180
                                                                   *
ATTCCGCTGA TCCATTTTCC GCCTTTTCCG GGTCTTTCAT CCCAAAGGGT ATCCTTTTCT

       190        200        210        220        230
ATCCTATCTT CTCAAAGGGT CAGTCAGTTC CCTCCA ATG CCT GCC GCC TCT TCC
                                        Met Pro Ala Ala Ser Ser>

     240              250              260              270              280
      *
CTG CTC GCT TCC CCT CTC TGT ACG TGG CTC CTT GCC GCC TGC ATG TCT
Leu Leu Ala Ser Pro Leu Cys Thr Trp Leu Leu Ala Ala Cys Met Ser>

       290              300              310              320              330
                         *
ACC TCC TTC CAC CCC TCC GAC CCT CTT CCG CCT TCC ATC TCC TCT CCT
Thr Ser Phe His Pro Ser Asp Pro Leu Pro Pro Ser Ile Ser Ser Pro>

           340              350              360              370
                                              *
CGC CGA CGC CTC TCC CGC CGC CGG ATT CTC TCC CAA TGC GCC CCA CTA
Arg Arg Arg Leu Ser Arg Arg Arg Ile Leu Ser Gln Cys Ala Pro Leu>
```

FIGURE 7
1/7

```
380         390         400         410         420
                                                  *
CCT TCT GCT TCC TCC GCC CTC CGC GGA TCC AGT TTC CAT ACC CTC GTC
Pro Ser Ala Ser Ser Ala Leu Arg Gly Ser Ser Phe His Thr Leu Val>

  430         440         450         460         470
ACC TCT TAC CTC GCC TGC TTC GAG CCC TGC CAT GAC TAC TAT ACA TCC
Thr Ser Tyr Leu Ala Cys Phe Glu Pro Cys His Asp Tyr Tyr Thr Ser>

    480         490         500         510         520
     *
GCA TCC TTG TTC GGA TCC AGA CCC ATT CGC ACC ACC CGC AGG CAC CGG
Ala Ser Leu Phe Gly Ser Arg Pro Ile Arg Thr Thr Arg Arg His Arg>

      530         540         550         560         570
                   *
AGG CTC AAT CGA GCT TCC CCT TCC AGG GAG GCA ATG GCC GTG GCT CTG
Arg Leu Asn Arg Ala Ser Pro Ser Arg Glu Ala Met Ala Val Ala Leu>

        580         590         600         610
                                 *
CAA CCT GAA CAG GAA GTT ACC ACA AAG AAG AAG CCA AGT ATC AAA CAG
Gln Pro Glu Gln Glu Val Thr Thr Lys Lys Lys Pro Ser Ile Lys Gln>

620         630         640         650         660
                                                  *
CGG CGA GTA GTT GTG ACT GGA ATG GGT GTG GTG ACT CCT CTA GGC CAT
Arg Arg Val Val Val Thr Gly Met Gly Val Val Thr Pro Leu Gly His>

  670         680         690         700         710
GAC CCT GAT GTT TTC TAC AAT AAT CTG CTT GAT GGA ACG AGT GGC ATA
Asp Pro Asp Val Phe Tyr Asn Asn Leu Leu Asp Gly Thr Ser Gly Ile>
```

FIGURE 7
2/7

```
     720           730           740           750           760
      *
AGC GAG ATA GAG ACC TTT GAT TGT GCT CAA TTT CCT ACG AGA ATT GCT
Ser Glu Ile Glu Thr Phe Asp Cys Ala Gln Phe Pro Thr Arg Ile Ala>

        770           780           790           800           810
                       *
GGA GAG ATC AAG TCT TTC TCC ACA GAT GGT TGG GTG GCC CCG AAG CTC
Gly Glu Ile Lys Ser Phe Ser Thr Asp Gly Trp Val Ala Pro Lys Leu>

           820           830           840           850
                                        *
TCT AAG AGG ATG GAC AAG TTC ATG CTA TAC ATG CTG ACC GCT GGC AAG
Ser Lys Arg Met Asp Lys Phe Met Leu Tyr Met Leu Thr Ala Gly Lys>

860          870           880           890           900
                                                        *
AAA GCA TTA ACA GAT GGT GGA ATC ACC GAA GAT GTG ATG AAA GAG CTA
Lys Ala Leu Thr Asp Gly Gly Ile Thr Glu Asp Val Met Lys Glu Leu>

  910           920           930           940           950
GAT AAA AGA AAA TGC GGA GTT CTC ATT GGC TCA GCA ATG GGT GGA ATG
Asp Lys Arg Lys Cys Gly Val Leu Ile Gly Ser Ala Met Gly Gly Met>

     960           970           980           990          1000
      *
AAG GTA TTC AAT GAT GCC ATT GAA GCC CTA AGG ATT TCA TAT AAG AAG
Lys Val Phe Asn Asp Ala Ile Glu Ala Leu Arg Ile Ser Tyr Lys Lys>

       1010          1020          1030          1040          1050
                       *
ATG AAT CCC TTT TGT GTA CCT TTC GCT ACC ACA AAT ATG GGA TCA GCT
Met Asn Pro Phe Cys Val Pro Phe Ala Thr Thr Asn Met Gly Ser Ala>
```

FIGURE 7
3/7

```
         1060        1070        1080        1090
                                    *
ATG CTT GCA ATG GAC TTG GGA TGG ATG GGG CCC AAC TAC TCG ATA TCT
Met Leu Ala Met Asp Leu Gly Trp Met Gly Pro Asn Tyr Ser Ile Ser>

1100        1110        1120        1130        1140
                                                   *
ACT GCT TGT GCA ACG AGT AAC TTT TGT ATA ATG AAT GCT GCG AAC CAT
Thr Ala Cys Ala Thr Ser Asn Phe Cys Ile Met Asn Ala Ala Asn His>

 1150        1160        1170        1180        1190
ATA ATC AGA GGC GAA GCA GAT GTG ATG CTT TGC GGG GGC TCA GAT GCG
Ile Ile Arg Gly Glu Ala Asp Val Met Leu Cys Gly Gly Ser Asp Ala>

   1200        1210        1220        1230        1240
     *
GTA ATC ATA CCT ATT GGT ATG GGA GGT TTT GTT GCA TGC CGA GCT TTG
Val Ile Ile Pro Ile Gly Met Gly Gly Phe Val Ala Cys Arg Ala Leu>

      1250        1260        1270        1280        1290
                    *
TCC CAG AGA AAT TCC GAC CCT ACT AAA GCT TCA AGA CCA TGG GAC AGT
Ser Gln Arg Asn Ser Asp Pro Thr Lys Ala Ser Arg Pro Trp Asp Ser>

         1300        1310        1320        1330
                                    *
AAT CGT GAT GGA TTT GTT ATG GGG GAA GGA GCT GGA GTG CTA CTA CTA
Asn Arg Asp Gly Phe Val Met Gly Glu Gly Ala Gly Val Leu Leu Leu>

1340        1350        1360        1370        1380
                                                   *
GAG GAG TTG GAG CAT GCA AAG AAA AGA GGT GCG ACT ATT TAC GCA GAA
Glu Glu Leu Glu His Ala Lys Lys Arg Gly Ala Thr Ile Tyr Ala Glu>
```

FIGURE 7
4/7

```
 1390          1400         1410          1420          1430
TTT CTA GGT GGG AGT TTC ACT TGC GAT GCC TAC CAC ATG ACC GAG CCT
Phe Leu Gly Gly Ser Phe Thr Cys Asp Ala Tyr His Met Thr Glu Pro>

   1440          1450         1460          1470          1480
      *
CAC CCT GAT GGA GCT GGA GTG ATT CTC TGC ATA GAG AAG GCT TTG GCT
His Pro Asp Gly Ala Gly Val Ile Leu Cys Ile Glu Lys Ala Leu Ala>

       1490          1500         1510          1520          1530
                        *
CAG TCA GGA GTC TCT AGG GAA GAC GTA AAT TAC ATA AAT GCC CAT GCC
Gln Ser Gly Val Ser Arg Glu Asp Val Asn Tyr Ile Asn Ala His Ala>

           1540          1550          1560          1570
                                          *
ACA TCC ACT CCG GCT GGA GAT ATC AAA GAG TAC CAA GCT CTT ATC CAC
Thr Ser Thr Pro Ala Gly Asp Ile Lys Glu Tyr Gln Ala Leu Ile His>

1580          1590          1600          1610          1620
                                                          *
TGT TTC GGC CAA AAC AGA GAG TTA AAA GTT AAT TCA ACC AAA TCA ATG
Cys Phe Gly Gln Asn Arg Glu Leu Lys Val Asn Ser Thr Lys Ser Met>

  1630          1640          1650          1660          1670
ATT GGT CAC CTT CTC GGA GCA GCC GGT GGT GTG GAA GCA GTT TCA GTA
Ile Gly His Leu Leu Gly Ala Ala Gly Gly Val Glu Ala Val Ser Val>

     1680          1690          1700          1710          1720
        *
GTT CAG GCA ATA AGG ACT GGG TGG ATC CAT CCG AAT ATT AAT TTG GAA
Val Gln Ala Ile Arg Thr Gly Trp Ile His Pro Asn Ile Asn Leu Glu>
```

FIGURE 7
5/7

```
        1730         1740          1750        1760        1770
                        *
AAC CCA GAT GAA GGC GTG GAT ACA AAA TTG CTC GTG GGT CCT AAG AAG
Asn Pro Asp Glu Gly Val Asp Thr Lys Leu Leu Val Gly Pro Lys Lys>

          1780        1790        1800         1810
                                     *
GAG AGA CTG AAC GTT AAG GTC GGT TTG TCT AAT TCA TTT GGG TTT GGT
Glu Arg Leu Asn Val Lys Val Gly Leu Ser Asn Ser Phe Gly Phe Gly>

1820        1830         1840        1850        1860       1870
                                                    *
GGG CAC AAC TCG TCC ATA CTC TTC GCC CCT TAC ATC TAG GAC GTTTCCGTGT
Gly His Asn Ser Ser Ile Leu Phe Ala Pro Tyr Ile ***>

       1880       1890       1900       1910       1920       1930
                                                      *
GTGGAATTCT ACTCAACATA TCAAAGCTGA AGTTTTGAGG ACTCCAGCAT GTTGGTAGCT

       1940       1950       1960       1970       1980       1990
                                                      *
CCTTACGTCT CTAGACATGC CCATGAGTTT TGTGTCCGGA GCTTTAGTCG GAACCATGAC

       2000       2010       2020       2030       2040       2050
                                                      *
GGATTGAGTA CTCATGGCGA CACTTGATAT ACTCCTTGCT AGAATTGTTG GTAGAGCAAT

       2060       2070       2080       2090       2100       2110
                                                      *
ATTCATTATC TCATATTTTT TTTTTCTCTG AAATCTCCCT CCTTGCAATA GTTGTACTTT

       2120       2130       2140       2150       2160       2170
                                                      *
CGAGCTTTTC ATCGAGTCAG TGAAGAAGAG AACAAAGCTG TTAACTCGGG CACGTAGTAA
```

FIGURE 7
6/7

```
      2180       2190       2200       2210       2220       2230
                                                     *
CCATTTGCCC TTTGTTTTGC TCTCTATTTC ATCACCGTTT TGTGGTTTTA AAATTTGTAA

      2240       2250       2260       2270       2280       2290
                                                     *
AACTAGAAGA CTGGTTTAGA TTGGTTTGTT TTCTCATTGA TAATTGGGGR ATGTATGTTT

      2300       2310       2320       2330       2340       2350
                                                     *
TGGAAATAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA

      2360
AGGGCGGCCG CTCTAGAGG
```

FIGURE 7
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Sequence Range: 1 to 2374

```
        10         20         30         40         50         60
                                                                 *
-A-CNTGGTC CGGAATTCCC GGGTCGACCC ACGCGTCCGC GACGCCAACC CACACCAAAC

        70         80         90        100        110        120
                                                                 *
TTCCTCAGCT TCTCTTCTCA AGACGGACGC CATTGGCAGC AGACAGACAG ACAGACAGAC

       130        140        150        160        170        180
                                                                 *
CCATAAAAGA GAGAGAGAGG GATCCATCGA ATGCGGCCAC CCTCCTTTCA TCTTCGATTC

       190        200        210        220        230        240
                                                                 *
ATTACCATAC CATTCCGCTG ATCCATTTTC CGCCTTTTCC GGGTCTTTCA TCCCAAAGGG

       250        260        270        280        290        300
                                                                 *
TATCCTTTTC TATCCTATCT TCTCAAAGGG TCAGTCAGTT CCCTCCAATG CCTGCCGCCT

       310        320        330        340        350        360
                                                                 *
CTTCCCTGCT CGCTTCCCCT CTCTGTACGT GGCTCCTTGC CGCCTGCATG TCTACCTCCT

       370        380        390        400        410        420
                                                                 *
TCCACCCCTC CGACCCTCTT CCGCCTTCCA TCTCCTCTCC TCGCCGACGC CTCTCCCGCC

       430        440        450        460        470        480
                                                                 *
GCCGGATTCT CTCCCAATGC GCCCCACTAC CTTCTGCTTC CTCCGCCCTC CGCGGATCCA
```

FIGURE 8
1/5

```
       490        500        510        520        530        540
                                                                 *
GTTTCCATAC CCTCGTCACC TCTTACCTCG CCTGCTTCGA GCCCTGCCAT GACTACTATA

       550        560        570        580        590        600
                                                                 *
CATCCGCATC CTTGTTCGGA TCCAGACCCA TTCGCACCAC CCGCAGGCAC CGGAGGCTCA

       610        620        630        640        650        660
                                                                 *
ATCGAGCTTC CCCTTCCAGG GGAGGCAATG GCCGTGGCTC TGCAACCTGA ACAGGAAGTT

       670        680        690        700        710        720
                                                                 *
ACCACAAAGA AGAAGCCAAG TATCAAACAG CGGCGAGTAG TTGTGACTGG AATGGGTGTG

       730        740        750        760        770        780
                                                                 *
GTGACTCCTC TAGGCCATGA ACCTGATGTT TTTCTACAAT AATCTGCTTG ATGGAACGAG

       790        800        810        820        830        840
                                                                 *
TGGCATAAGC GAGATAGAGA CCTTTGATTG TGCTCAATTT CCTACGAGAA TTGCTGGAGA

       850        860        870        880        890        900
                                                                 *
GATCAAGTCT TTCTCCACAG ATGGTTGGGT GGCCCCGAAG CTCTCTAAGA GGATGGACAA

       910        920        930        940        950        960
                                                                 *
GTTCATGCTA TACATGCTGA CTGCTGGCAA GAAAGCATTA ACAGATGGTG GAATCACCGA

       970        980        990       1000       1010       1020
                                                                 *
AGATGTGATG AAAGAGCTAG ATAAAAGAAA ATGCGGAGTT CTCATTGGCT CAGCAATGGG
```

FIGURE 8
2/5

```
      1030       1040       1050       1060       1070       1080
                                                                *
TGGAATGAAG GTATTCAATG ATGCCATTGA AGCCCTAAGG ATTTCATATA AGAAGATGAA

      1090       1100       1110       1120       1130       1140
                                                                *
TCCCTTTTGT GTACCTTTCG CTACCACAAA TATGGGATCA GCTATGCTTG CAATGGACTT

      1150       1160       1170       1180       1190       1200
                                                                *
GGGATGGATG GGGCCCAACT ACTCGATATC TACTGCTTGT GCAACGAGTA ACTTTTGTAT

      1210       1220       1230       1240       1250       1260
                                                                *
AATGAATGCT GCGAACCATA TAATCAGAGG CGAAGCAGAT GTGATGCTTT GCGGGGGCTC

      1270       1280       1290       1300       1310       1320
                                                                *
AGATGCGGTA ATCATACCTA TTGGTATGGG AGGTTTTGTT GCATGCCGAG CTTTGTCCCA

      1330       1340       1350       1360       1370       1380
                                                                *
GAGAAATTCC GACCCTACTA AAGCTTCAAG ACCATGGGAC AGTAATCGTG ATGGATTTGT

      1390       1400       1410       1420       1430       1440
                                                                *
TATGGGGGAA GGAGCTGGAG TGCTACTACT AGAGGAGTTG GAGCATGCAA AGAAAAGAGG

      1450       1460       1470       1480       1490       1500
                                                                *
TGCGACTATT TACGCAGAAT TTCTAGGTGG GAGTTTCACT TGCGATGCCT ACCACATGAC
```

FIGURE 8
3/5

```
      1510       1520       1530       1540       1550       1560
                                                                *
CGAGCCTCAC CCTGATGGAG CTGGAGTGAT TCTCTGCATA GAGAAGGCTT TGGCTCAGTC

      1570       1580       1590       1600       1610       1620
                                                                *
AGGAGTCTCT AGGGAAGACG TAAATTACAT AAATGCCCAT GCCACATCCA CTCCGGCTGG

      1630       1640       1650       1660       1670       1680
                                                                *
AGATATCAAA GAGTACCAAG CTCTTATCCA CTGTTTCGGC CAAAACAGAG AGTTAAAAGT

      1690       1700       1710       1720       1730       1740
                                                                *
TAATTCAACC AAATCAATGA TTGGTCACCT TCTCGGAGCA GCCGGTGGTG TGGAAGCAGT

      1750       1760       1770       1780       1790       1800
                                                                *
TTCAGTAGTT CAGGCAATAA GGACTGGGTG GATCCATCCG AATATTAATT TGGAAAACCC

      1810       1820       1830       1840       1850       1860
                                                                *
AGATGAAGGC GTGGATACAA AATTGCTCGT GGGTCCTAAG AAGGAGAGAC TGAACGTTAA

      1870       1880       1890       1900       1910       1920
                                                                *
GGTCGGTTTG TCTAATTCAT TTGGGTTTGG TGGGCACAAC TCGTCCATAC TCTTCGCCCC

      1930       1940       1950       1960       1970       1980
                                                                *
TTACATCTAG GACGTTTCGT GTGTGGAATT CTACTCAACA TATCAAAGCT GAAGTTTTGA

      1990       2000       2010       2020       2030       2040
                                                                *
GGACTCCAGC ATGTTGGTAG CTCCTTACGT CTCTAGACAT GCCCATGAGT TTTGTGTCCG
```

FIGURE 8
4/5

```
       2050       2060       2070       2080       2090       2100
                                                                  *
GAGCTTTAGT CGGAACCATG ACGGATTGAG TACTCATGGC GACACTTGAT ATACTCCTTG

       2110       2120       2130       2140       2150       2160
                                                                  *
CTAGAATTGT TGGTAGAGCA ATATTCATTA TCTCATATTT TTTTTTTCTC TGAAATCTCC

       2170       2180       2190       2200       2210       2220
                                                                  *
CTCCTTGCAA TAGTTGTACT TTCGAGCTTT TCATCGAGTC AGTGAAGAAG AGAACAAAGC

       2230       2240       2250       2260       2270       2280
                                                                  *
TGTTAACTCG GGCACGTAGT AACCATTTGC CCTTTGTTTT GCTCTCTATT TCATCACCGT

       2290       2300       2310       2320       2330       2340
                                                                  *
TTTGTGGTTT TAAAATTTGT AAAACTAGAA GACTGGTTTA GATTGGTTTG TTTTCTCAAA

       2350       2360       2370
AAAAAAAAAA AAGGGCGGCC GCTCTAGAGG ATCC
```

FIGURE 8
5/5

```
Sequence Range: 1 to 1580

           10         20         30         40         50
CCTGAATCGG ATTCAAGAGA GAGTTTCGTT GCTGGG ATG GCG AAT GCA TCT GGG
                                        Met Ala Asn Ala Ser Gly>

      60         70         80         90         100
       *
TTT CTG GGT TCT TCA GTT CCT GCC CTG AGA AGG GCA ACT CAG CAT TCG
Phe Leu Gly Ser Ser Val Pro Ala Leu Arg Arg Ala Thr Gln His Ser>

       110         120         130         140         150
                     *
ATT TCA TCG TCT CGT GGA TCT TCC TCG GAG TTT GTC TCC AAA AGG GTG
Ile Ser Ser Ser Arg Gly Ser Ser Ser Glu Phe Val Ser Lys Arg Val>

         160         170         180         190
                                   *
TTT TGC TGT AGT GCC GTT CAG GAT TCT GAC AGG CAG TCT TTG GGT GAT
Phe Cys Cys Ser Ala Val Gln Asp Ser Asp Arg Gln Ser Leu Gly Asp>

200         210         220         230         240
                                                  *
TCT CGC TCG CCG AGG CTT GTG AGT AGA GGA TGC AAA TTA ATT GGA TCT
Ser Arg Ser Pro Arg Leu Val Ser Arg Gly Cys Lys Leu Ile Gly Ser>

  250         260         270         280         290
GGT TCT GCT ATA CCA GCT CTT CAA GTC TCA AAT GAT GAT CTT GCT AAA
Gly Ser Ala Ile Pro Ala Leu Gln Val Ser Asn Asp Asp Leu Ala Lys>

    300         310         320         330         340
     *
ATT GTC GAC ACC AAT GAT GAA TGG ATT ACT GTC CGA ACG GGG ATC CGC
Ile Val Asp Thr Asn Asp Glu Trp Ile Thr Val Arg Thr Gly Ile Arg>
```

FIGURE 9
1/5

```
         350         360         370         380         390
                      *
AAC CGA AGG GTT CTC TCA GGT AAA GAT AGT CTT ACA AAT TTA GCA TCA
Asn Arg Arg Val Leu Ser Gly Lys Asp Ser Leu Thr Asn Leu Ala Ser>

            400         410         420         430
                                     *
GAG GCA GCA AGG AAA GCT CTA GAG ATG GCA CAG GTA GAC GCA AAT GAT
Glu Ala Ala Arg Lys Ala Leu Glu Met Ala Gln Val Asp Ala Asn Asp>

440         450         460         470         480
                                                  *
GTG GAT ATG GTT TTG ATG TGT ACT TCT ACC CCT GAG GAC CTT TTC GGC
Val Asp Met Val Leu Met Cys Thr Ser Thr Pro Glu Asp Leu Phe Gly>

 490         500         510         520         530
AGT GCT CCT CAG ATA TCG AAA GCA CTT GGC TGC AAA AAG AAT CCT TTG
Ser Ala Pro Gln Ile Ser Lys Ala Leu Gly Cys Lys Lys Asn Pro Leu>

   540         550         560         570         580
    *
TCT TAC GAC ATT ACC GCT GCA TGC AGT GGA TTT GTG TTG GGT TTA GTC
Ser Tyr Asp Ile Thr Ala Ala Cys Ser Gly Phe Val Leu Gly Leu Val>

      590         600         610         620         630
                   *
TCA GCT GCT TGC CAC ATT AGA GGT GGG GGT TTT AAC AAT ATT CTA GTG
Ser Ala Ala Cys His Ile Arg Gly Gly Gly Phe Asn Asn Ile Leu Val>

         640         650         660         670
                                  *
ATT GGT GCT GAT TCT CTT TCT CGG TAT GTT GAC TGG ACC GAT CGG GGA
Ile Gly Ala Asp Ser Leu Ser Arg Tyr Val Asp Trp Thr Asp Arg Gly>
```

FIGURE 9
2/5

```
680          690          700          710          720
                                                    *
ACA TGT ATT CTC TTT GGA GAT GCT GCT GGA GCT GTA GTG GTG CAG TCA
Thr Cys Ile Leu Phe Gly Asp Ala Ala Gly Ala Val Val Val Gln Ser>

  730          740          750          760          770
TGT GAT GCT GAG GAA GAT GGG CTC TTT GCT TTT GAT TTG CAT AGC GAT
Cys Asp Ala Glu Glu Asp Gly Leu Phe Ala Phe Asp Leu His Ser Asp>

    780          790          800          810          820
     *
GGA GAT GGG CAA AGG CAT CTA AAA GCT GCA ATC AAA GAA GAT GAA GTT
Gly Asp Gly Gln Arg His Leu Lys Ala Ala Ile Lys Glu Asp Glu Val>

       830          840          850          860          870
                     *
GAT AAA GCC CTG GGA CAT AAT GGG TCC ATC AGA GAT TTT CCA CCA AGG
Asp Lys Ala Leu Gly His Asn Gly Ser Ile Arg Asp Phe Pro Pro Arg>

          880          890          900          910
                                     *
CGT TCT TCA TAC TCT TGC ATC CAA ATG AAC GGT AAA GAG GTA TTC CGC
Arg Ser Ser Tyr Ser Cys Ile Gln Met Asn Gly Lys Glu Val Phe Arg>

920          930          940          950          960
                                                     *
TTT GCT TGC CGC TCT GTG CCT CAG TCA ATC GAA TCA GCA CTT GGA AAG
Phe Ala Cys Arg Ser Val Pro Gln Ser Ile Glu Ser Ala Leu Gly Lys>

  970          980          990          1000         1010
GCC GGT CTT AAT GGA TCC AAC ATC GAC TGG TTG CTG CTT CAT CAG GCA
Ala Gly Leu Asn Gly Ser Asn Ile Asp Trp Leu Leu Leu His Gln Ala>
```

FIGURE 9
3/5

```
 1020       1030       1040       1050       1060
   *
AAT CAG AGG ATC ATT GAT GCA GTA GCA ACA CGT CTA GAG GTT CCT CAA
Asn Gln Arg Ile Ile Asp Ala Val Ala Thr Arg Leu Glu Val Pro Gln>

     1070       1080       1090       1100       1110
                   *
GAA CGA ATT ATC TCA AAC TTG GCA AAT TAC GGG AAC ACT AGT GCG GCA
Glu Arg Ile Ile Ser Asn Leu Ala Asn Tyr Gly Asn Thr Ser Ala Ala>

        1120       1130       1140       1150
                                 *
TCC ATT CCC TTG GCA CTA GAC GAA GCT GTG AGG AGT GGA AAT GTG AAG
Ser Ile Pro Leu Ala Leu Asp Glu Ala Val Arg Ser Gly Asn Val Lys>

1160       1170       1180       1190       1200
                                               *
CCG GGT CAC GTG ATT GCA ACC GCA GGA TTT GGC GCC GGA CTC ACA TGG
Pro Gly His Val Ile Ala Thr Ala Gly Phe Gly Ala Gly Leu Thr Trp>

  1210       1220       1230       1240       1250       1260
                                                            *
GGT TCT GCT ATT ATC AGG TGG GGA TAA GACTGAA GCCGAGCCAG CACTGCAGCT
Gly Ser Ala Ile Ile Arg Trp Gly ***>

      1270       1280       1290       1300       1310       1320
                                                               *
TCCTCTCAAA CCGATGTTTC ACGAAATTTT GCTTCCATGA CCANAAAAAG AAGAAGTCAG

      1330       1340       1350       1360       1370       1380
                                                               *
TCTTTTATGG AGCAAGCAAC ACGACACGAT CTTCATCACA TTGCCCTTTT TCGTTCCCCT
```

FIGURE 9

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```
       1390       1400       1410       1420       1430       1440
                                                                 *
TTTCCATTAG TTTGATGATT TTGCTGACAA TACAATACCC ATAGTTTCTT TTGTCCCCAA

       1450       1460       1470       1480       1490       1500
                                                                 *
TAAGTTATTT GTTTCTTGTT TAATTGTTCA GCTTTTACTT CATTTTGTCT CGGGACATTG

       1510       1520       1530       1540       1550       1560
                                                                 *
GAGATGACAG CATAAACATC ATGTTTATAT TTTGCTAAAA AAAAAAAAAA AAAAAAAAAA

       1570       1580
AAAAAAAAAA AAAAAAAAAA
```

FIGURE 9
5/5

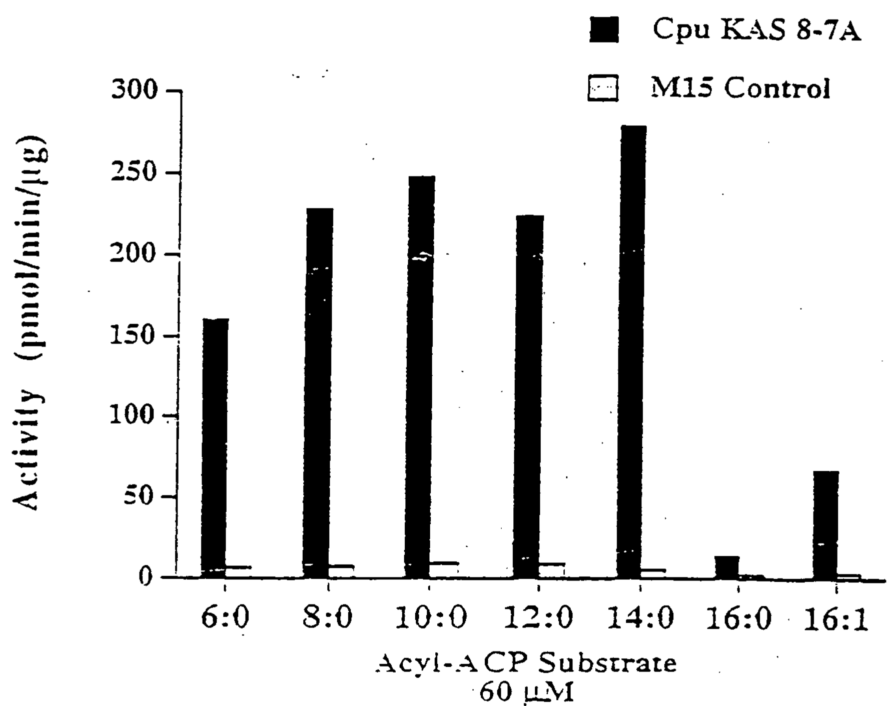
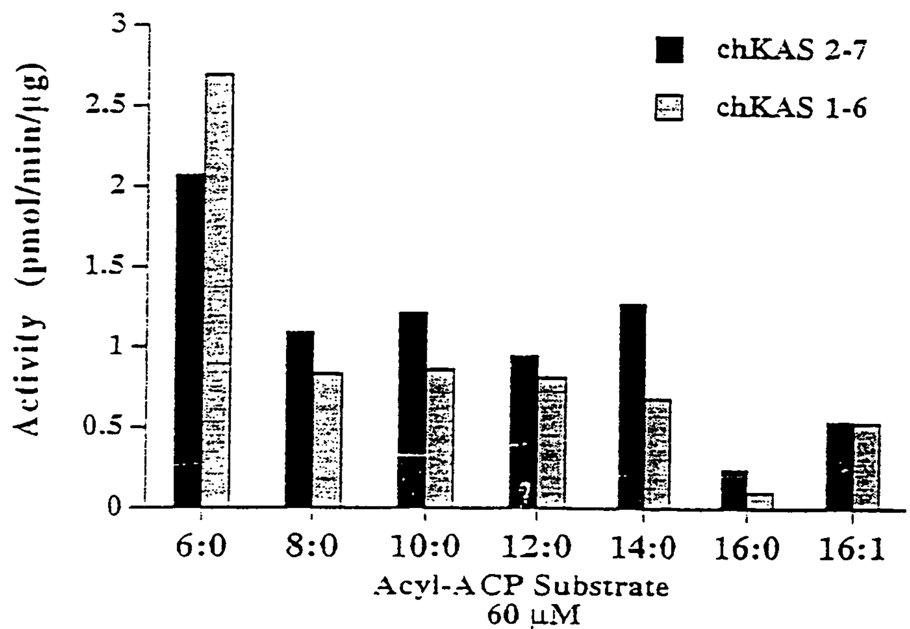

Cpu KAS 8-7A
M15 Control
Activity (pmol/min/μg)
300
250
200
150
100
50
0
6:0
8:0
10:0
12:0
14:0
16:0
16:1
Acyl-ACP Substrate
60 μM

FIGURE 10



3
2.5
2
1.5
1
0.5
0
Activity (pmol/min/µg)
chKAS 2-7
chKAS 1-6
6:0
8:0
10:0
12:0
14:0
16:0
16:1
Acyl-ACP Substrate
60 µM

FIGURE 11

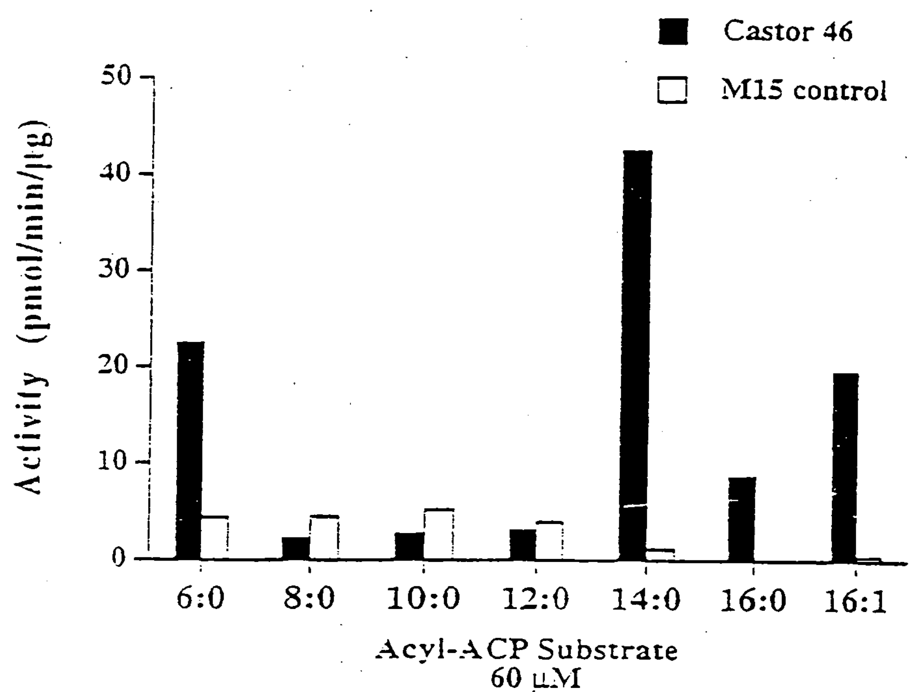
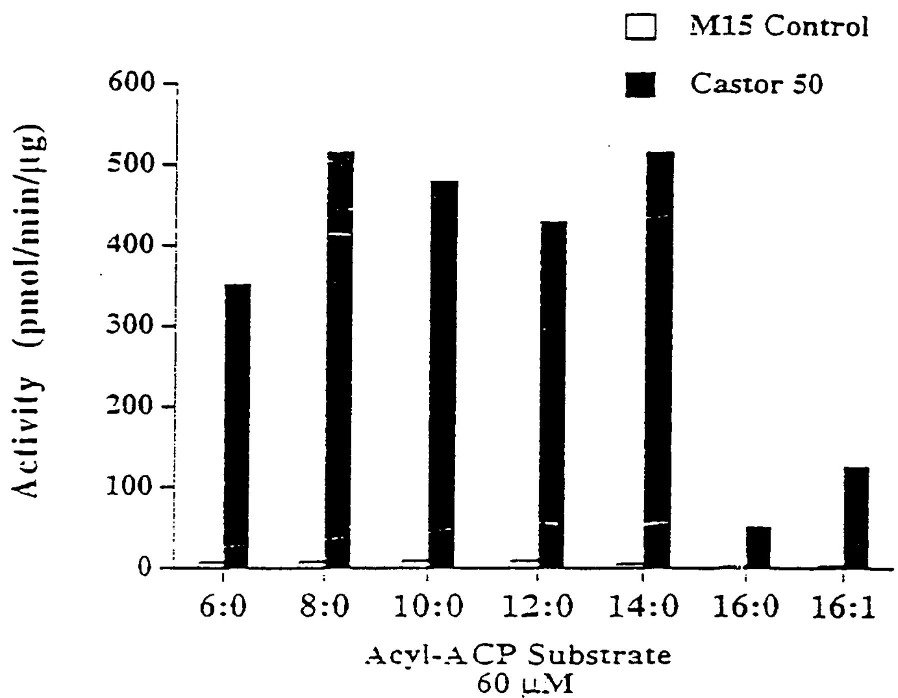
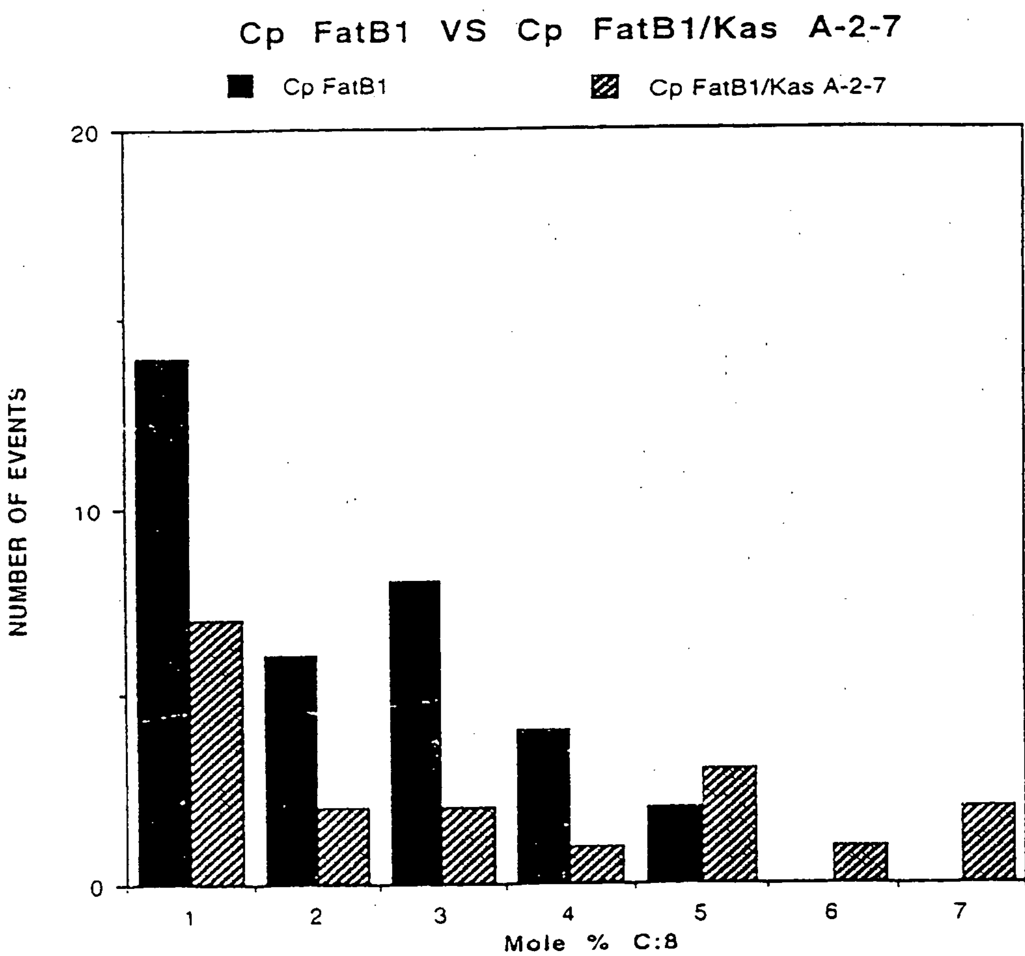

Castor 46
M15 control
Activity (pmol/min/µg)
50
40
30
20
10
0
6:0
8:0
10:0
12:0
14:0
16:0
16:1
Acyl-ACP Substrate
60 µM

FIGURE 12



E328013-28

FIGURE 13



Cp FatB1 VS Cp FatB1/Kas A-2-7
Cp FatB1
Cp FatB1/Kas A-2-7
NUMBER OF EVENTS
20
10
0
1
2
3
4
5
6
7
Mole % C:8

FIGURE 14

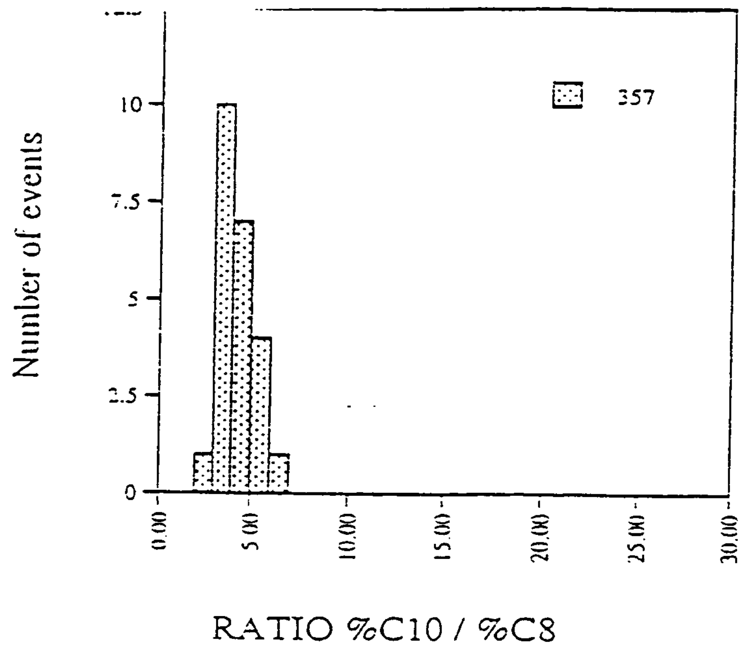

357
Number of events
10
7.5
5
2.5
0
0.00
5.00
10.00
15.00
20.00
25.00
30.00
RATIO %C10 / %C8

FIGURE 15
1/2

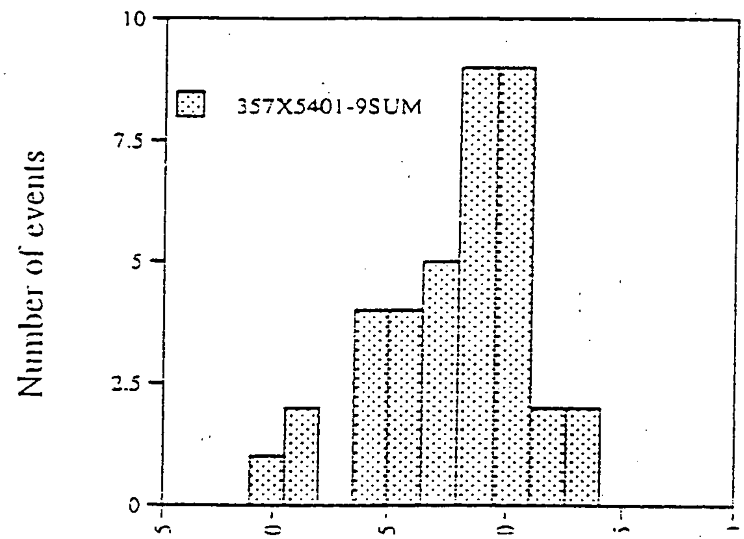


FIGURE 15
2/2

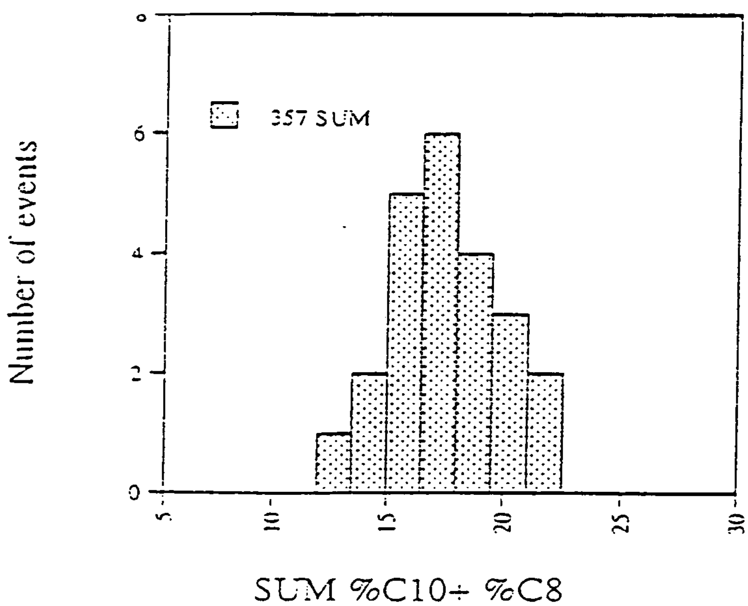


FIGURE 16

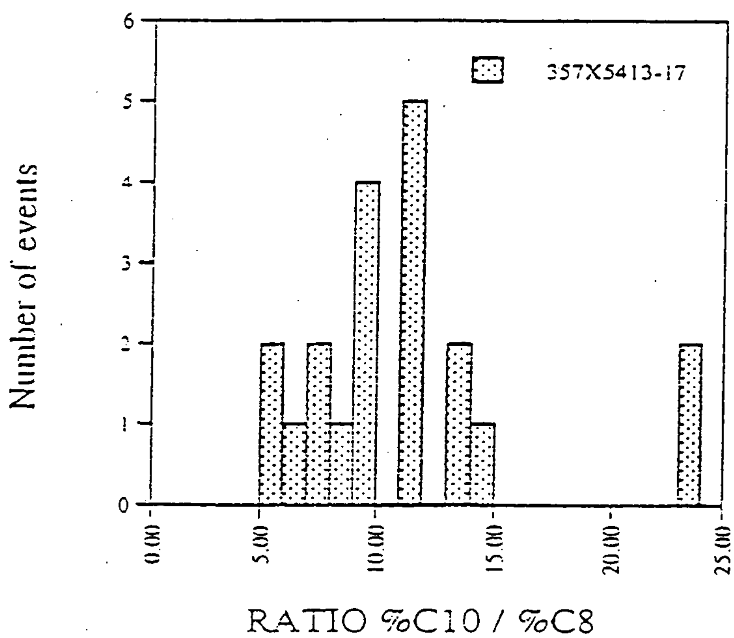

357X5413-17
Number of events
0
1
2
3
4
5
6
0.00
5.00
10.00
15.00
20.00
25.00
RATIO %C10 / %C8

FIGURE 17
1/2

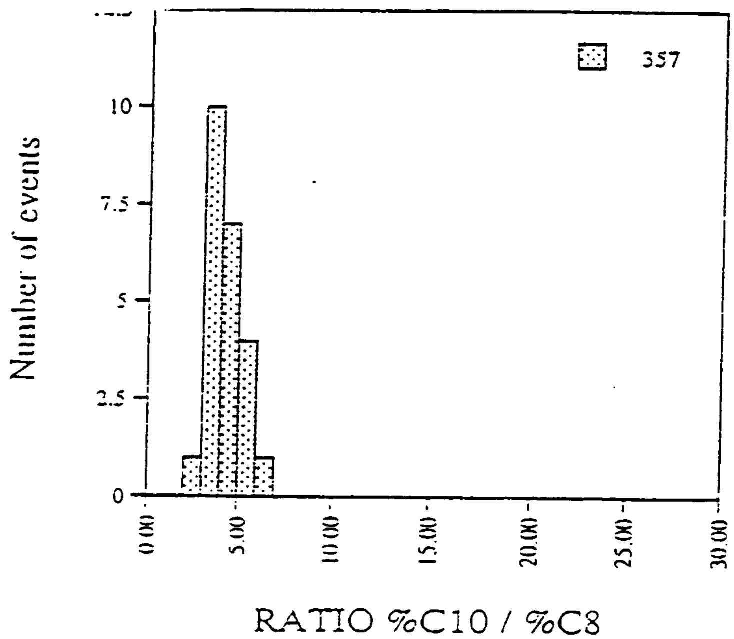

357
10
7.5
5
2.5
0
Number of events
0.00
5.00
10.00
15.00
20.00
25.00
30.00
RATIO %C10 / %C8

FIGURE 17

2/2

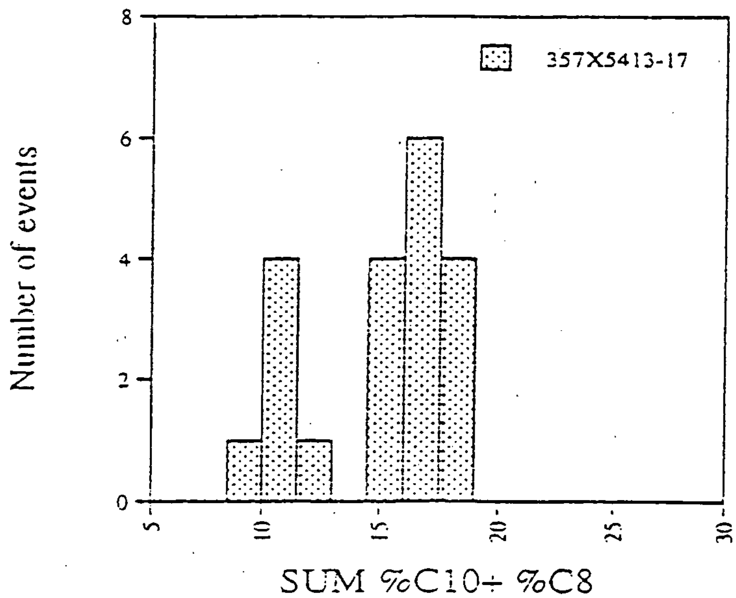


FIGURE 18
1/2

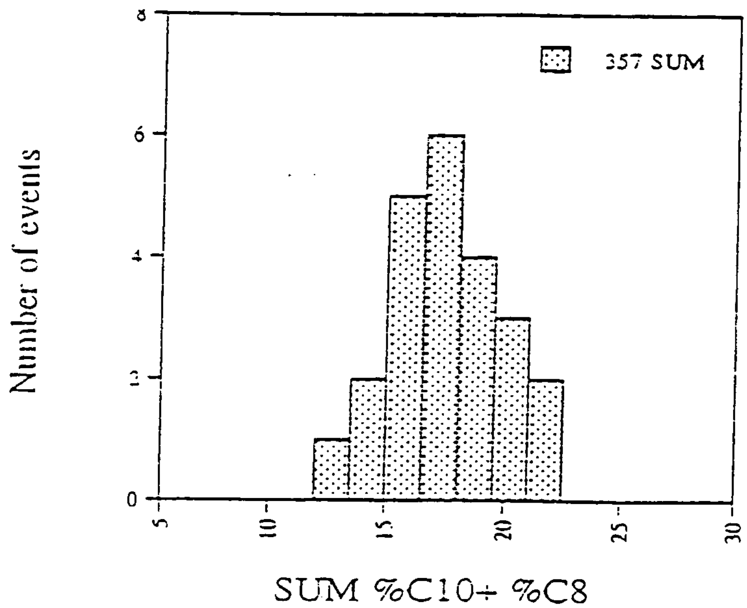
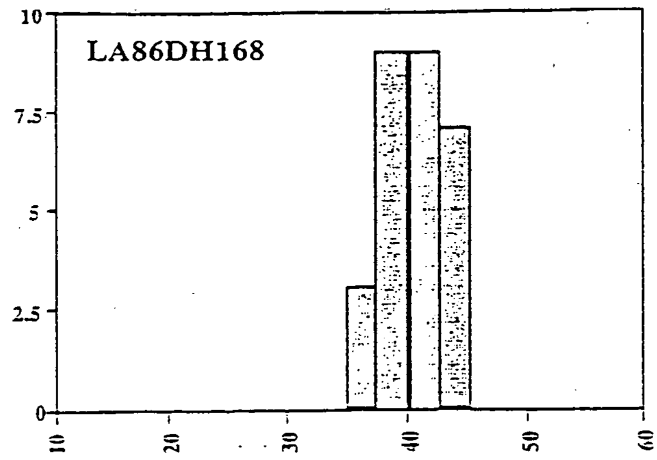


FIGURE 18
2/2



Number of independent events

12:0 levels (w%)

FIGURE 19
1/3

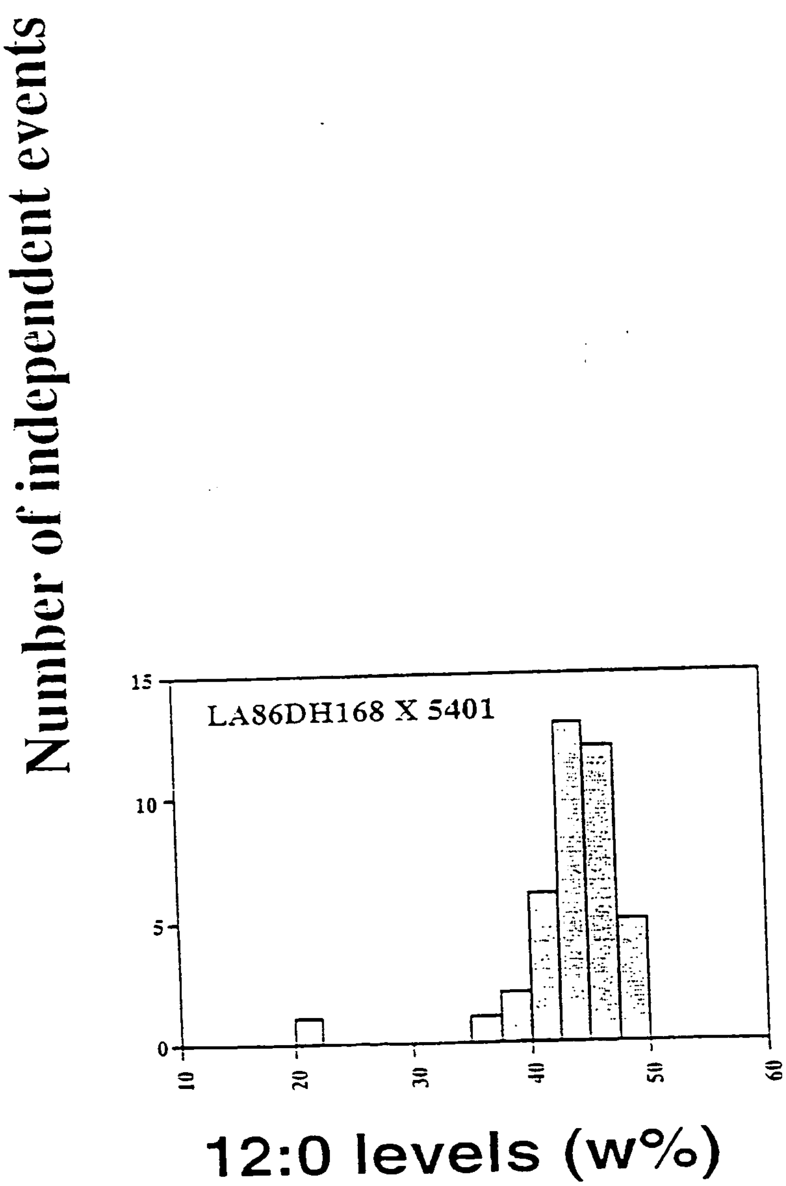

Number of independent events
LA86DH168 X 5401
15
10
5
0
10
20
30
40
50
60
12:0 levels (w%)

FIGURE 19
2/3

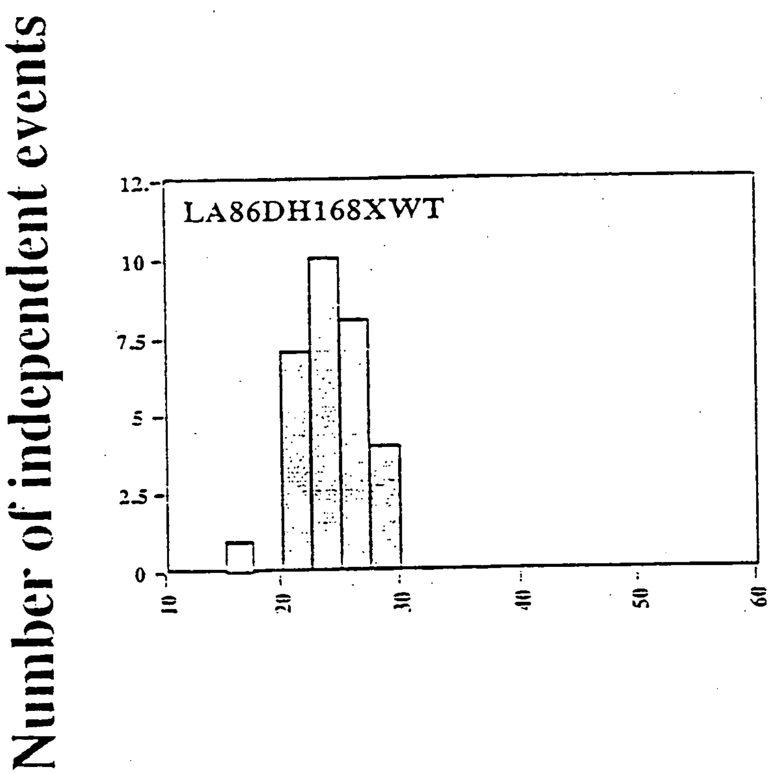
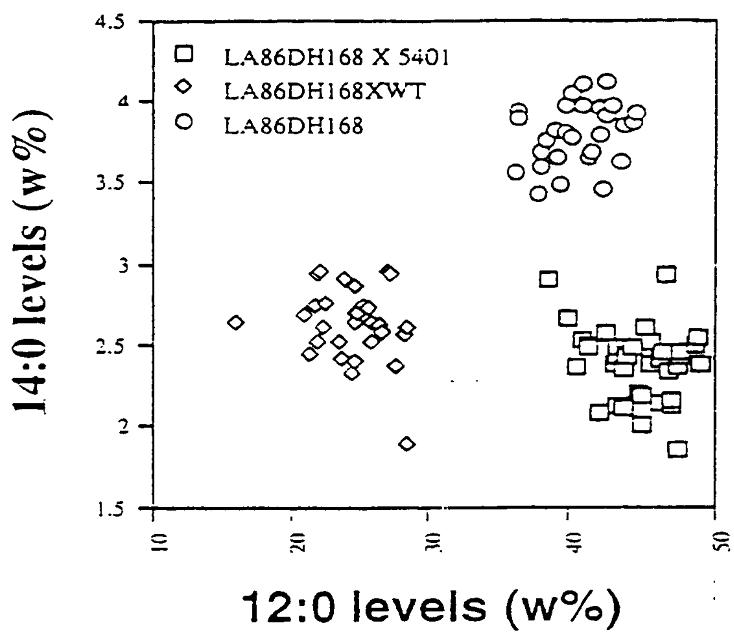

Number of independent events
LA86DH168XWT
12
10
7.5
5
2.5
0
10
20
30
40
50
60

FIGURE 19
3/3



4.5
4
3.5
3
2.5
2
1.5
14:0 levels (w%)
LA86DH168 X 5401
LA86DH168XWT
LA86DH168
10
20
30
40
50
12:0 levels (w%)

FIGURE 20

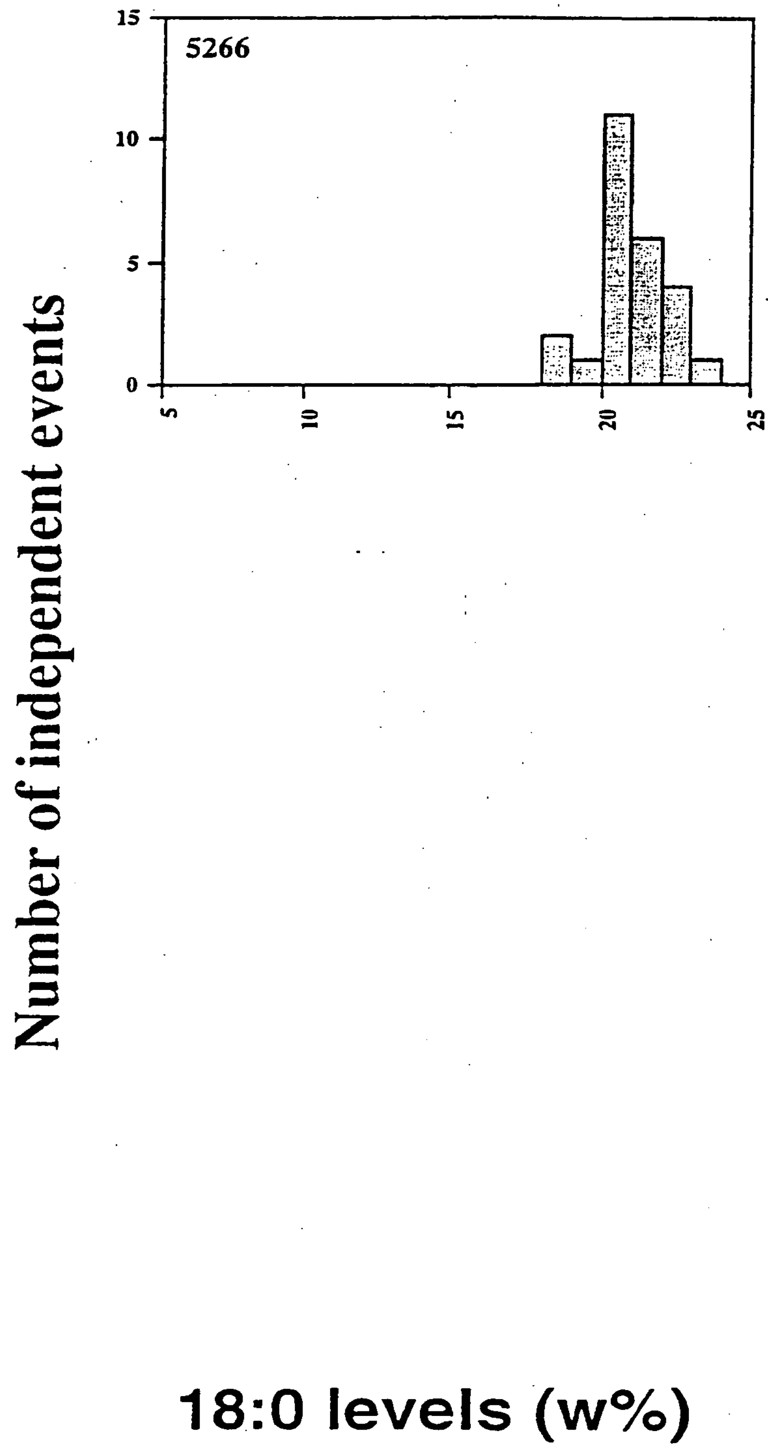

5266
15
10
5
0
5
10
15
20
25
Number of independent events
18:0 levels (w%)

FIGURE -21

1/3

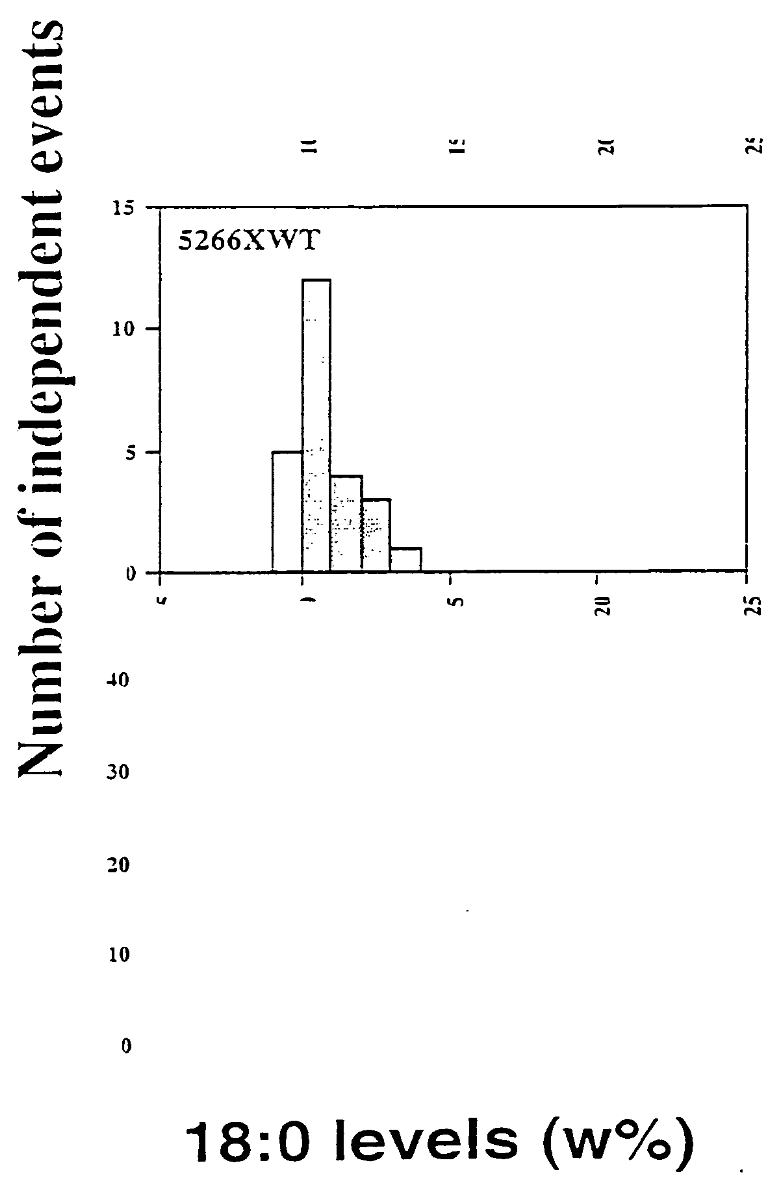

Number of independent events
5266XWT
15
10
5
0
40
30
20
10
0
18:0 levels (w%)

FIGURE 21
2/3

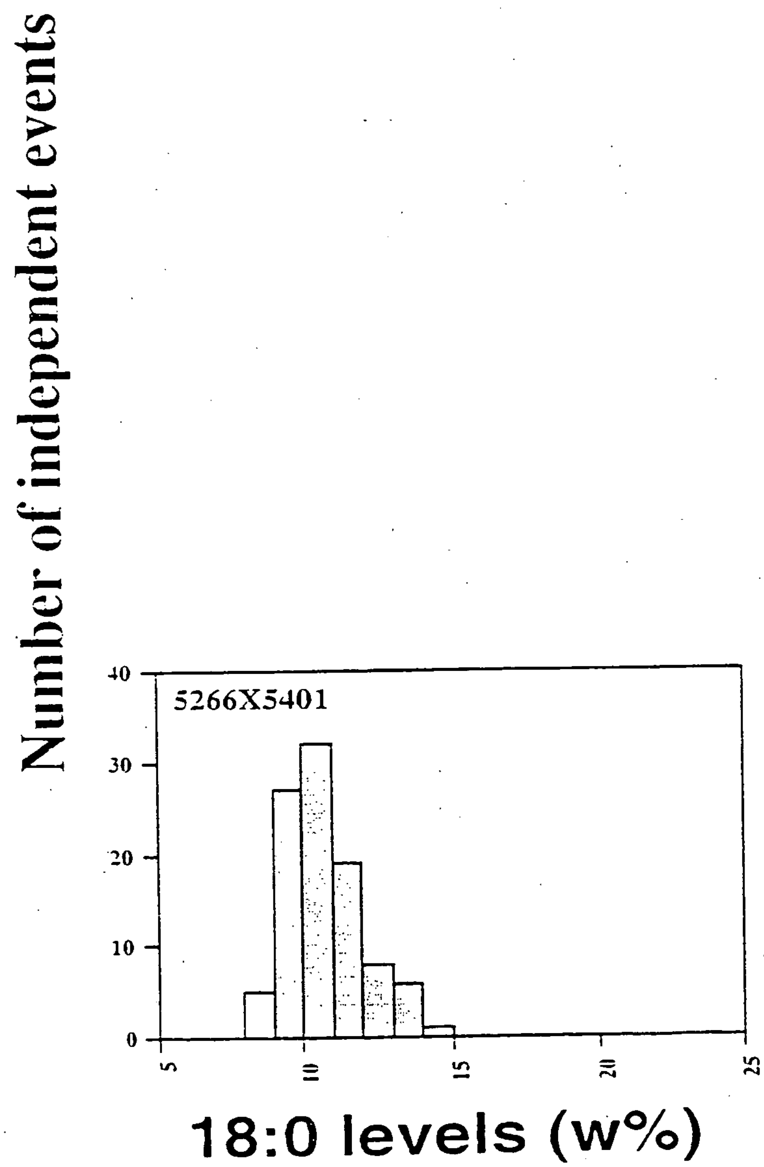

Number of independent events
5266X5401
40
30
20
10
0
5
10
15
20
25
18:0 levels (w%)

FIGURE 21
3/3

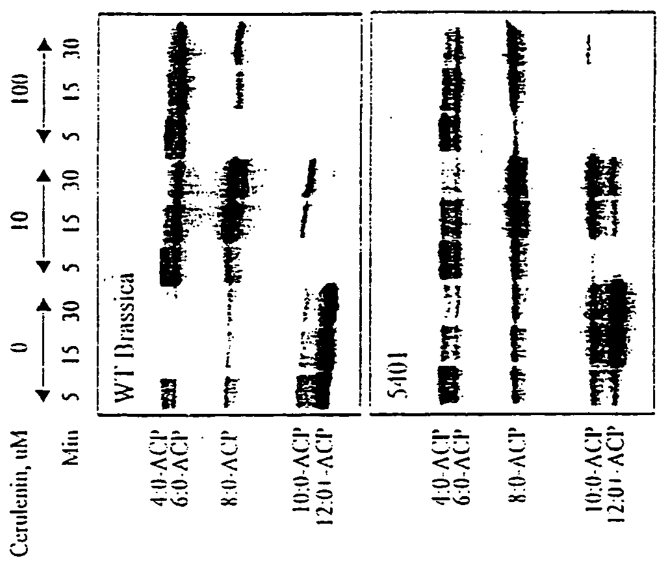

Cerulenin, uM
0
10
100
Min
5 15 30 5 15 30 5 15 30
WT Brassica
4:0-ACP
6:0-ACP
8:0-ACP
10:0-ACP
12:0-ACP
5401
4:0-ACP
6:0-ACP
8:0-ACP
10:0-ACP
12:0-ACP

FIGURE 22

INTERNATIONAL SEARCH REPORT

Inte. .ional Application No
PCT/US 98/07114

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/82 C12N15/54

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimumdocumentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 06740 A (MAX PLANCK GESELLSCHAFT ;TOEPFER REINHARD (DE); MARTINI NORBERT (D) 9 March 1995 see page 16, paragraph 1; claim 17 ---	15,22
P,X	LEONARD, J.M., ET AL.: "A Cuphea beta-ketoacyl-ACP synthase shifts the synthesis of fatty acids towards shorter chains in Arabidopsis seeds expressing Cuphea FatB thioesterases" THE PLANT JOURNAL, vol. 13, no. 5, March 1998, pages 621-628, XP002081429 see the whole document ---	15,22, 29-32

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publicationdate of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of theinternational search	Date of mailing of the international search report
20 October 1998	02/11/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Maddox, A

1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	DEHESH, K., ET AL.: "Production of hgh levels of 8:0 and 10:0 fatty acids in transgenic canola by overexpression of Ch FatB2, a thioesterase from Cuphea hookeriana" THE PLANT JOURNAL, vol. 9, no. 2, 1996, pages 167-172, XP002081430 see page 170, column 2, paragraph 2 ---	15,22
A	DEHESH K ET AL: "TWO NOVEL THIOESTERASES ARE KEY DETERMINANTS OF THE BIMODAL DISTRIBUTION OF ACYL CHAIN LENGTH OF CUPHEA PALUSTRIS SEED OIL" PLANT PHYSIOLOGY, vol. 110, 1996, pages 203-210, XP002014020 see page 209, column 2, paragraph 2 ---	15,22
A	SLABAUGH, M.B., ET AL.: "Cuphea wrightii beta-ketoacyl-ACP synthase II (CwKASII1) mRNA complete cds." EMBL SEQUENCE ACCESSION NO. U67317, 13 December 1996, XP002081431 see the whole document ---	15,22
A	FUHRMANN, J., ET AL.: "Factors controlling medium-chain fatty acid synthesis in plastids from maturing Cuphea embryos" Z. NATURFORSCH., vol. 48c, 1993, pages 616-622, XP002081432 see the whole document ---	15,22
A	SCHUCH, R., ET AL.,: "Medium-chain acyl-ACP thioesterase is not the exclusive enzyme responsible for early chain-length termination in medium-chain fatty acid synthesis" GRASAS Y ACEITES, vol. 44, 1993, pages 126-128, XP002081433 see the whole document ---	15,22
A	TOEPFER R ET AL: "MODIFICATION OF PLANT LIPID SYNTHESIS" SCIENCE, vol. 268, 5 May 1995, pages 681-685, XP002014017 see page 684, column 3, paragraph 2 ---	15,22
A	MARTINI N ET AL: "MODIFICATION OF FATTY ACID COMPOSITION IN THE STORAGE OIL OF TRANSGENIC RAPESEED" BIOLOGICAL CHEMISTRY HOPPE-SEYLER, vol. 376, September 1995, page S55 XP002014021 ---	15,22
	-/--	

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/07114

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94 10189 A (CALGENE INC) 11 May 1994 see page 5, line 34 - line 36 ---	15,22
A	WO 92 03564 A (CALGENE INC) 5 March 1992 see page 22, line 19 - line 23 ---	15,22
A	WO 96 23892 A (CALGENE INC ;DEHESH KATAYOON (US); VOELKER TONI ALOIS (US); HAWKIN) 8 August 1996 see the whole document ---	15,22
A	WO 95 13390 A (CALGENE INC ;VOELKER TONI ALOIS (US); YUAN LING (US); KRIDL JEAN () 18 May 1995 see the whole document ---	15,22
A	WO 94 10288 A (CALGENE INC ;VOELKER TONI ALOIS (US); DAVIES HUW MAELOR (US); KNUT) 11 May 1994 see the whole document ---	15,22
A	WO 92 20236 A (CALGENE INC) 26 November 1992 see the whole document ---	15,22
A	VOELKER, T.A., ET AL.: "Genetic engineering of a quantitative trait: metabolic and genetic parameters influencing the accumulation of laurate in rapeseed." THE PLANT JOURNAL, vol. 9, no. 2, 1996, pages 229-241, XP002081434 see the whole document ---	15,22
A	WO 93 10240 A (DU PONT) 27 May 1993 see the whole document ---	15,22
A	WO 95 15387 A (CALGENE INC ;METZ JAMES GEORGE (US); LARDIZABAL KATHRYN DENNIS (US) 8 June 1995 see the whole document ---	15,22
T	DEHESH, K., ET AL.: "KAS IV: a 3-ketoacyl-ACP synthase form Cuphea sp. is a medium chain specific condensing enzyme" THE PLANT JOURNAL, vol. 15, no. 3, August 1998, pages 383-390, XP002081435 see the whole document -----	15,22, 29-32

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/07114

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

International Application No. PCT/ US 98 /07114

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Claims Nos.: 1-14,19,20,21,26,27,28

Remark : Claims 1-14 were not provided to the ISA at the time of search and hence the subject matter of these claims and the dependent claims 19,20, 21,26,27,28, could not be defined.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 98/07114

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9506740 A	09-03-1995	AU 688377 B	12-03-1998
		AU 7739894 A	22-03-1995
		CA 2169094 A	09-03-1995
		EP 0716708 A	19-06-1996
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		CA 2212003 A	08-08-1996
		EP 0807182 A	19-11-1997
WO 9513390 A	18-05-1995	CA 2176137 A	18-05-1995
		EP 0728212 A	28-08-1996
		JP 9505470 T	03-06-1997
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		CA 2147617 A	11-05-1994
		EP 0670903 A	13-09-1995
		JP 8502892 T	02-04-1996
		US 5654495 A	05-08-1997
		US 5667997 A	16-09-1997
WO 9220236 A	26-11-1992	US 5512482 A	30-04-1996
		CA 2109580 A	26-11-1992
		EP 0557469 A	01-09-1993
		US 5639790 A	17-06-1997
		US 5455167 A	03-10-1995
		JP 7501924 T	02-03-1995
WO 9310240 A	27-05-1993	AU 3073592 A	15-06-1993

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte. ional Application No
PCT/US 98/07114

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9310240 A		CA 2123495 A	27-05-1993
		EP 0667906 A	23-08-1995
		JP 7501446 T	16-02-1995
		MX 9206540 A	01-05-1993
		US 5500361 A	19-03-1996
WO 9515387 A	08-06-1995	US 5679881 A	21-10-1997
		CA 2177598 A	08-06-1995
		EP 0731840 A	18-09-1996
		JP 9505739 T	10-06-1997

Form PCT/ISA/210 (patent family annex) (July 1992)